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With four Plates, and one hundred and eighty-two Figures

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# A New Interpretation of Coniferous Cones

## I. Podocarpaceae (Podocarpus)

BY

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With forty Figures in the Text

### INTRODUCTION

THE interpretation of coniferous cones is one of the oldest unsolved problems of comparative morphology. Investigation has centred in general on attempts to explain the diversified nature of the ovulate cone and to homologize it in some way with the staminate. Theories exist based on anatomical, teratological, and developmental evidence. Although such theories are numerous, none has been wholly satisfactory. Up to the present time a lack of fossil evidence has been keenly felt, and even when such evidence has been available the difficulties of interpretation have added to the confusion. With the recently published findings of Florin (1939) among Palaeozoic fossils, however, the problem now seems possible of solution. Florin's work not only clarifies our existing evidence, but suggests, as for the present investigation, the direction in which further research may yield results.

From Florin's reinterpretation of the cordaite fructifications it is certainly now clear that both the male and female consisted of fertile branches composed of secondary units (fertile dwarf-shoots), simple in organization and similar in structure. What have generally been considered small, compound female strobili, until Schoute (1925) and Eltringham (1936) offered the first suggestions to the contrary, Florin has found to be the secondary simple units of a fertile branch, homologous with those of the male (Fig. 7). These fertile branches then, considered as a whole, were compound; the units of which they were composed simple, secondary, determinate axes, which bore spirally arranged sterile and fertile appendages. The ovules and the sporangia were borne, as in the Devonian Psilophytes, in a terminal position on the primitively dichotomously branched fertile appendages (Fig. 8, Aa, Ba). In certain Palaeozoic conifers the female fertile branch was compacted into a cone (Fig. 8, C), but the organization of the secondary units still closely resembled the dwarf-shoots of *Cordaianthus* (Fig. 8, Ca). Furthermore, Florin has demonstrated a series in reduction among fossil conifers which involves progressive flattenings and fusions among the appendages of the female dwarf-shoot and leads convincingly to the greatly reduced seed-scale complex of modern conifers (Fig. 8).

The problem has now assumed new aspects, and several important conclusions may be drawn from Florin's work. First, confirmation from fossils of the shoot nature of the ovuliferous scale in modern conifers now exists. The steps of reduction and fusion are clearly indicated in fossil forms, not only fusion among the members of the fertile dwarf-shoot, but fusion of the subtending bract to the seed-scale complex. This latter fact should offer proof additional to that of the anatomical facts for such families as the Araucariaceae, Podocarpaceae, Taxodiaceae, and Cupressaceae.

Second, Florin's critical study of the primitive fertile dwarf-shoot necessitates a reinterpretation of the ovuliferous scale in present-day families. It is now evident that the fertile or ovuliferous scale itself as it exists in modern forms is the remnant of this short axis with its fused sterile and fertile appendages. The ovules of the conifers can no longer be considered borne on the dorsal or ventral side of a leaf as in the filicinean or lycopodinian series. The fertile appendages in the cordaite line bore terminal sporangia on ultimate segments which had been ancestrally specialized in that function, as in the Devonian Psilophytes. It must be concluded then that the ovules in the present-day conifers are terminal on fertile appendages of the disappearing shoot which are now fused with similar sterile appendages to form the ovuliferous scale (Fig. 8, Fa). Existing developmental evidence certainly supports this view (Herzfeld, 1914; Goebel, 1933). The number of fertile appendages on the dwarf-shoots had already been reduced in the Palaeozoic conifers from the larger number in the cordaites. As Florin has pointed out, in lines leading to the present-day families, undoubtedly the number and position of the remaining fertile appendages were determining characters. In reduction the seed-scale complex with distal fertile appendages persisting (Fig. 8, Cb) would certainly assume a structure different from that in which the basal appendages were fertile and the distal ones sterile (Fig. 8, Cc). Likewise the erect or inverted position of the ovule (with probable exceptions) are characters inherited from Palaeozoic families. The inverted position may, in some forms, have resulted from the fusion of recurved fertile and sterile appendages into the cone unit.

Third, in the Cordaitales both the male and female fertile branches ('inflorescences') were compound, but, as Florin has pointed out, the Palaeozoic conifers, although possessing compound female inflorescences similar in organization to those of the cordaites, had, so far as known, male cones which had already become simple in structure (Fig. 8, D, Da). The question naturally arises: What changes have occurred in the male fructifications? Such changes in the Palaeozoic conifers must have occurred before the Carboniferous. Nothing in the known fossil record has left us transitions from the cordaite condition to the coniferous. In turning to living forms we find again a characteristic prevalence of simple male cones. However, in the Podocarpaceae, a large coniferous family of the Southern Hemisphere, two species of *Podocarpus* are characterized by similar male and female inflorescences (Figs. 1-6, 8 E, H). In these two species the ovuliferous scale

(epimatium) completely encloses a single ovule (Fig. 2), but the seed-scale complexes are not organized into cones; they are borne on fertile branches in the axils of bracts separated by internodes. Similar fertile branches bear small male cones, relatively unreduced dwarf-shoots, in the axils of bracts likewise separated by internodes. Undoubtedly these two species have retained very primitive characteristics. It was, therefore, believed that a study of the male cone arrangement in this family would yield important facts as a basis for interpreting the evolutionary changes that have led to the present position of the simple coniferous male cone. Evidence will be presented to show that the male cone is a simple, unreduced, secondary, determinate axis, homologous with the primitive female dwarf-shoot, which in its present form is reduced to the seed-scale complex. The primary fertile branch of the female has generally been compacted into a cone; the primary fertile branch of the male has suffered reduction and elimination of units (Fig. 8). The steps in this process have been preserved within the Podocarpaceae.

#### MATERIAL, METHODS, AND TERMINOLOGY

The material for the present study was almost entirely obtained from herbarium specimens.<sup>1</sup> Cones were boiled until soft and examinations were made under a Spencer binocular dissecting microscope. In a few cases anatomical sections were made, but, in general, study of external features by dissection was found to be sufficient.

Since there is great variety in the terminology of various authors, the terms used in the present study have been defined. In some instances new terms have been employed; in others, old ones have been retained.

*Primary fertile branch.* A specialized branch bearing determinate, secondary fertile shoots subtended by bracts.

*Fertile branch unit.* The secondary, determinate fertile shoot borne axillary to a bract of the primary fertile branch. (It is thought advisable to drop the term 'dwarf-shoot' in connexion with the reproductive structure because its usage suggests an homology with the vegetative dwarf-shoots of the Pinaceae, which is not now supported by fossil evidence.)

*Bract.* The foliar appendage of the primary fertile branch which subtends a secondary fertile branch unit.

<sup>1</sup> This was made possible through the kindness of the authorities of the New York Botanical Garden, the Arnold Arboretum and the Gray Herbarium of Harvard University, the Herbarium of the University of California, the Bailey Hortorium and the Herbarium of Cornell University. For preserved material I am indebted to Professor M. A. Chrysler of Rutgers University, to Professor E. W. Sinnott of Yale University, and to Professor A. J. Eames of Cornell University. Fresh material of *Sciadopitys* was kindly sent me by Professor Herbert Wahl of Pennsylvania State College. To Professor R. B. Thomson of the University of Toronto I am also greatly indebted for habit and anatomical drawings of *Austrotaxus spicata*. The identification of herbarium material where possible was always checked by taxonomic descriptions and by comparison of several specimens. For assistance in identifications and other kindnesses I am grateful to Dr. Robert T. Clausen of Cornell University and to Dr. Leon Croizat of the Arnold Arboretum.



**Scale leaf.** A leaf of which the lamina is reduced. The term may include bud scales, but excludes the above-defined bract.

**Primary cluster.** An aggregate of fertile branch units (male cones), brought together by a greater or lesser reduction in internodes of one primary fertile branch.

**Secondary cluster.** An aggregate of fertile branches (each generally reduced to a single unit), brought together by a greater or lesser reduction in the internodes of a secondary vegetative branch on which the fertile branches are borne.

**Tertiary cluster.** An aggregate of secondary clusters, brought together by a greater or lesser reduction in the internodes of a primary vegetative branch.

**Peduncle.** The naked stalk of a cluster of staminate cones or the naked stalk below the fleshy female receptacle, notably in the Eupodocarpus section of the genus Podocarpus.

**Pedicel.** The stalk of an individual cone in a secondary or tertiary cluster, representing the reduced primary fertile branch.

#### DESCRIPTION OF PODOCARPUS

The Podocarpaceae are a large family distributed chiefly in the Southern Hemisphere. Of the seven genera in the family Podocarpus is the largest, with sixty to seventy species, many of which are endemic to the region in which they are found. The genus as a whole has a wide distribution.

In most members of the family an epimatium surrounds the ovule as a more or less fleshy, berry-like structure. The identity of the epimatium as a modified ovuliferous scale has long been recognized through the work of Sinnott (1913), Herzfeld (1914), and Aase (1915). It is clear also from Florin's fossil evidence that this axillary structure represents a much reduced shoot, the fertile and sterile members of which have become greatly modified and fused.

Pilger (1926) has divided Podocarpus into sub-groups, based mainly upon the manner in which the ovules are borne. In the subgenus Stachycarpus the ovules are carried in spikes or as one or two at the end of branchlets and no receptacle is formed at maturity. The second subgenus, Protopodocarpus, characterized mostly by axillary ovules which are generally borne on a fleshy receptacle formed at maturity by the fusion of bract bases, is divided again into several sections.

The Nageia section has wide, many-nerved leaves; the Eupodocarpus section, linear or lanceolate leaves; the Microcarpus section, small scale leaves. These three have an epimatium entirely free from the subtending bract ('carpel' in Pilger's terminology). The bract does not equal the epimatium in length.

The Dacrycarpus section, with very small leaves, is characterized by fusion of the bract with the epimatium; the bract equals the epimatium in length.

## EVOLUTION OF THE MALE FERTILE BRANCH

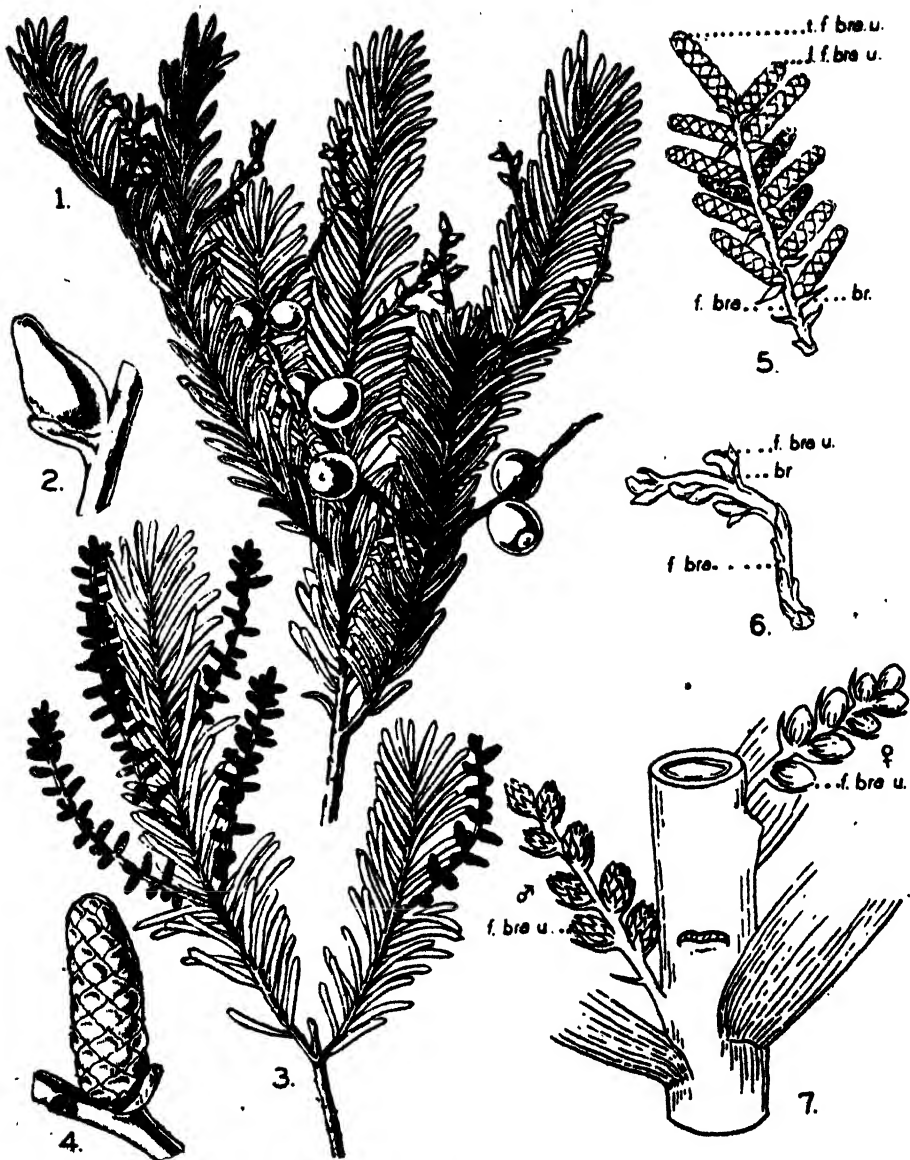
*The primitive fertile branch.*

Two species, *P. spicatus* R. Br. and *P. andinus* Poeppig., belonging to the *Stachycarpus* section of the genus, are remarkable in that they bear an undoubtedly primitive type of fertile branch. *P. spicatus*, the matai, is a dioecious tree endemic in New Zealand. Both the ovules and small staminate cones are spirally arranged on separate fertile branches (Figs. 1-4). The male fertile branches vary in length from 1 or 2 to 4 or 5 cm.; correspondingly, they vary in the number of bracts and axillary fertile branch units. Pilger (1926, p. 219) gives ten to thirty. An apical bud may terminate this branch, but frequently there is a terminal male cone, usually surrounded at its base by crowded bracts whose axils are sterile. Serial sections through this region show the cone to be truly terminal. The vascular bundles of the branch continue into the cone axis with little change. At the base of the fertile branch are bud scales or scars of bud scales similar to those at the base of the vegetative branches. Above them on all the herbarium specimens examined are scars of a few apparently sterile bracts, separated by internodes gradually increasing in length upward from the bud scales.

The small male cones are sessile (Fig. 4) and bear neither bracts nor bud scales on their axes, with the exception of the above-mentioned terminal unit which is surrounded at the base by sterile bracts. The sporophylls extend to the very base of the cone axis; the two or three lowest may sometimes be sterile or bear only one sporangium instead of the usual two. They are always identical with the fertile sporophylls and are without question similar structures. They bear no resemblance to the larger single bract which subtends the cone.

The bracts of the fertile branch resemble in form but not in size the vegetative leaves. Like the leaves also the base of the bract is fused to the branch. Those which subtend fertile branch units, either male or female, are only a few millimetres in length, considerably shorter than the vegetative leaves. Generally, the fertile branches are axillary to vegetative leaves. They may, however, be formed in a terminal position on vegetative branches. In such a case, according to Pilger (1926, p. 219), a gradual transition in size occurs from the vegetative leaves to the fertile bracts. I found no such transition in the herbarium material at my disposal. The terminal fertile branches are all limited from the vegetative by the presence of crowded bud-scale scars. No case was found, however, in which this short sterile region was lacking at the base of the branch.

*P. andinus*, the 'plum-fruited yew', is one of the few monoecious forms in the genus. Like *P. spicatus* it has a limited distribution and is found wild only in the Andes of southern Chile, where it is endemic. It was formerly known under the name of *Prumnopitys elegans* Philippi. The small, sessile male cones (Fig. 5) are arranged, as in *P. spicatus*, in the axils of spirally placed bracts on fertile branches. In most of the material examined a terminal cone is present.



FIGS. 1-7. Figs. 1-4. *Podocarpus spicatus*. After Hooker (1843). Fig. 1. Habit, showing female fertile branches bearing young ovules or mature fruit. Slightly reduced. Fig. 2. Single female fertile branch unit (young ovule enclosed in its epimatium) enlarged, in the axil of a bract. Fig. 3. Habit, showing male fertile branches bearing small sessile male cones. Slightly reduced. Fig. 4. Single male fertile branch unit (male cone) enlarged, in the axil of a bract. Figs. 5-6. *Podocarpus andinus*. Fig. 5. Male fertile branch (*f.bra.*) bearing lateral fertile branch units (*l.f.bra.u.*), each in the axil of a bract (*br.*) and a terminal fertile branch unit (*t.f.bra.u.*) surrounded at the base by several sterile bracts. ( $\times 2$ .) Fig. 6. Female fertile branch (*f.bra.*) bearing fertile branch units (*f.bra.u.*) each in the axil of a bract (*br.*). ( $\times 2$ .) Fig. 7. *Cordaites laevis*. Redrawn from Grand'Eury (1877). As in the two preceding *Podocarpus* species, both male and female fertile branches bear simple fertile branch units (*f.bra.u.*), secondary fertile shoots axillary to bracts. Slightly enlarged.

Similarly, the cones bear no bracts on their axes; two sterile sporophylls are usually present which resemble the fertile ones. A few sterile bracts are at the base of the fertile branch above the bud scales. The bracts of this species are sometimes larger than those of *P. spicatus*, although Pilger (1926, p. 219) states that they never reach the foliage leaves in length.

Two other species, whose position in the *Nageia* section of the genus is questionable, show interesting modifications in their fertile branches. *P. minor* (Carr.) Parl. is endemic in New Caledonia where it occurs at an altitude of 3,500 ft. (Compton, 1922). The male cones (Fig. 29) are generally solitary and terminal on short, leafy branches. Below the terminal cone are, constantly, six thick, fleshy, broad bracts in decussate pairs, the upper four lighter in colour (in dried specimens) and with obtuse apices. The lowest pair are more narrow, longer, darker, and may possibly be reduced leaves. Below these bracts are vegetative leaves, the two uppermost a little smaller than the others but considerably larger than the bracts. At the base of this cone-bearing branch, which is on an average of 3 to 4 cm. in length, the leaves decrease gradually in size but the internodes between them are not shortened and no bud scales are present. The bases of both leaves and bracts are decurrent, those of the four upper bracts more thickened and fleshy. The size, position, and number of these bracts below all the terminal cones was found to be constant. One specimen was found to have, in addition to the terminal cone, single, sessile cones axillary to three of the bracts.

Five or six examples were also found of solitary cones on short, bracteate stalks axillary to vegetative leaves. The stalks, however, bear eight to ten nearly opposite bracts under the cone, the upper four always lighter in colour (in dried specimens) and broader. Since the number of bracts associated with cones terminal on leafy branches is constantly six, the presence of more bracts on these cones axillary to vegetative leaves would indicate that they are also terminal on much shortened branches on which two to four of the supposed basal bracts are in reality reduced leaves. On one of these shortened branches another example was found in which the two upper bracts also bear sessile axillary cones. The cones axillary to bracts never bear anything but sporophylls on their axes.

The distribution of *P. vitiensis* Seem.—limited to Fiji—suggests, as for *P. minor*, that it is the remnant of an old flora which had once a much wider distribution. Unfortunately I was not able to obtain male cones of this species, but reasonably accurate conclusions as to their organization may be drawn from an excellent illustration accompanying Seeman's description of the species (1865, Tab. LXXVII). Gibbs (1909, p. 182), who saw the tree growing in Fiji, has also described the cones. Their terminal position at the end of leafy branches is apparently closely similar to *P. minor*. Vegetative characters are also somewhat similar in the two species. In fact one can easily imagine *P. minor* to be *P. vitiensis* grown under more severe climatic conditions.

Although the limits of a year's growth are difficult to determine on speci-

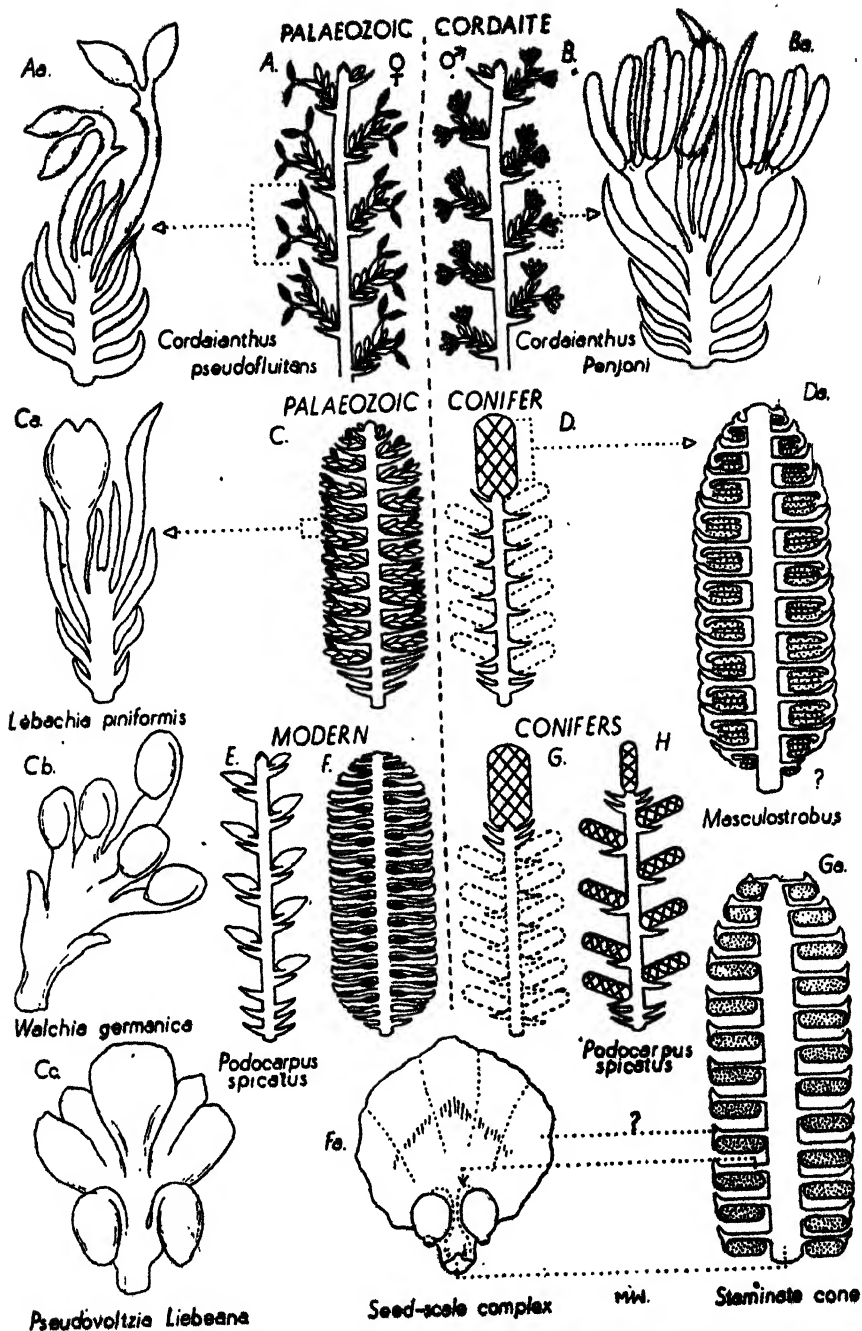


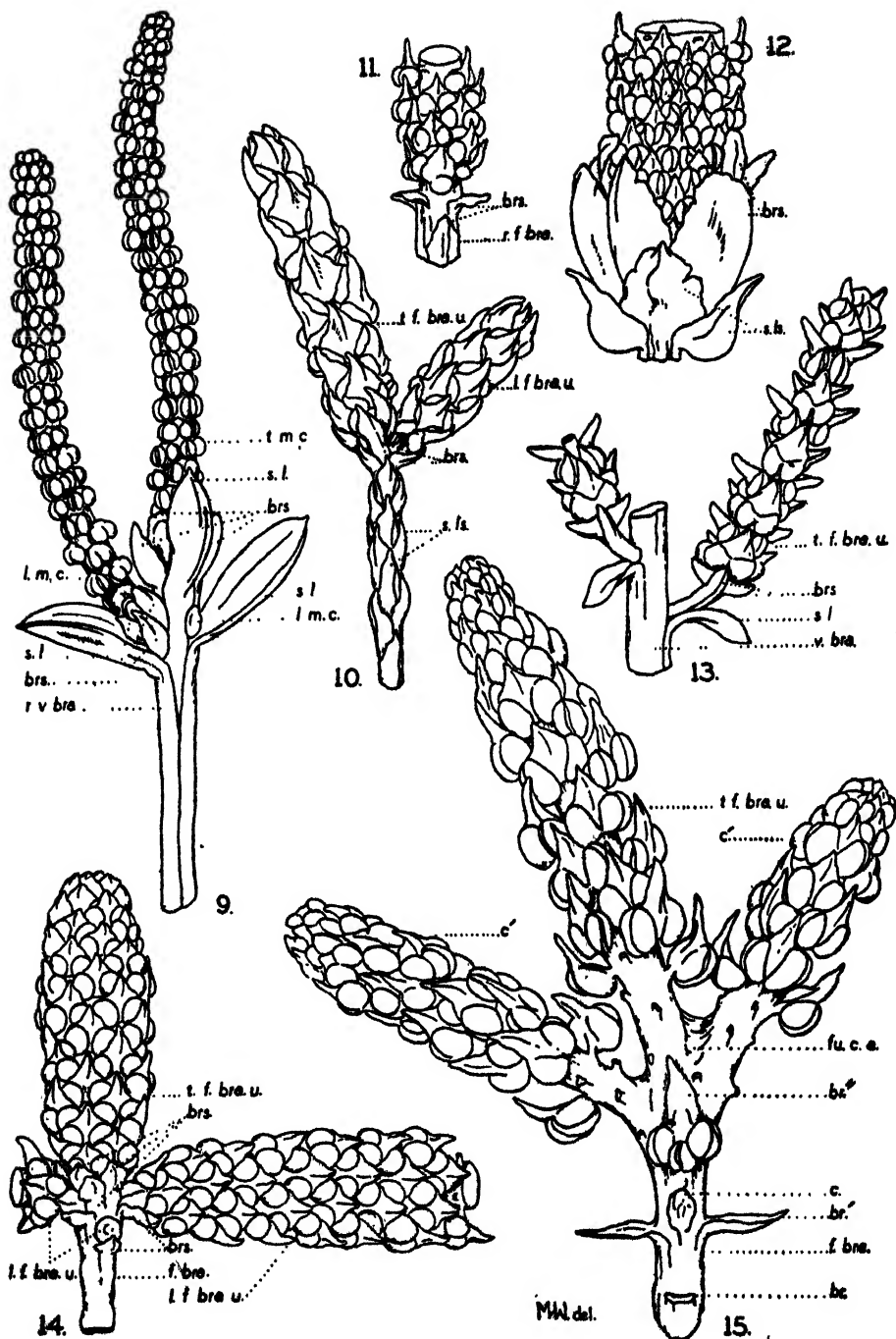
FIG. 8. Diagrams showing the evolution of coniferous cones. Aa, Ca, Cb, and Cc are based on photographs by Florin (1939) of fossil secondary fertile shoots. Da is based on a description by Florin (1928) of *Masculostrobus harrissowitsi*. The dotted lines in D and G represent lost structures; those in Fa represent fusion between parts; the dotted lines between the seed-scale complex Fa and the staminate cone Ga indicate homologous structures. For a complete explanation see the text.

mens from the tropics, all the evidence seems to point to the fact that in both these species the entire branch on which the cones are terminal is homologous with the fertile branches of *P. spicatus*, both borne laterally on vegetative branches (Fig. 39). It is possible that in a few cases in which the cone-bearing branch is found to be longer than the others that it is borne in a terminal position on an already existent vegetative branch. Such cases occur in *P. spicatus*. In the majority, however, the cone-bearing branches are lateral and appear to correspond to a season's growth. If this is the case, *P. minor* and *P. vitiensis* are characterized by a type of fertile branch somewhat different from that in *P. spicatus*. In the last species the greater part of the branch is fertile; in the two former a basal vegetative portion exists with a limited terminal fertile region. Reduction has certainly occurred in the loss of fertile branch units axillary to bracts, only a terminal one generally persisting. It seems probable also that the extent of the fertile portion has also been reduced to the six or possibly four remaining upper bracts. The number of fertile bracts in *P. spicatus* is indeterminate. The opposite to subopposite arrangement of both bracts and leaves in *P. minor* indicates branch shortening. The shortening has, however, been uniform. It is an interesting fact that neither *P. minor* nor *P. vitiensis* shows bud scales. Although the base of the vegetative branches as well as the fertile branches bear smaller leaves, the internodes here are not shorter than elsewhere on the branches. In *P. spicatus*, on the other hand, the fertile region is probably unreduced, but the fact that the base of the fertile branch is characterized by bud scales and a few sterile bracts with shortened internodes may indicate a reduction in a basal vegetative portion. It is possible, therefore, that the primitive male fertile branch (Fig. 39) may originally have consisted of both a distal fertile region and a proximal vegetative one.

#### *Primary clusters and single cones.*

Early steps in the reduction of the primitive fertile branch involved not only a loss of the basal vegetative region but a shortening of internodes in the fertile region, with the result that the fertile branch units were brought together into loose primary clusters. A loss also in number of units generally accompanied the clustering. Several species of the *Stachycarpus* group and *Nageia* section are found to be at this stage of evolution.

In *P. amarus* Blume two to five male cones are borne axillary to bracts on fertile branchlets (Fig. 14). By reduction of internodes between the bracts the cones are in loose primary clusters. Inserted towards the apex of the fertile branchlet, 6 or 7 mm. in length, are two or three, usually opposite, close pairs of broad, divergent bracts, their decurrent bases partly sheathing the branchlet. A cone is usually borne in the axil of each of the lowest two bracts, with a third cone terminal on the branchlet and surrounded by two to four opposite sterile bracts. The cones are sessile and may have two or more sterile sporophylls at the base which generally resemble the fertile



FIGS. 9-15. Fig. 9. *P. nivalis*. Secondary cluster of male cones. The reduced vegetative branch (*r.v.bra.*) bears two decussate pairs of scale leaves (*s.l.*) surrounding a cluster of three fertile branches, each reduced to a single cone or unit bearing sterile bracts (*brs.*) at the base. One reduced fertile branch is terminal (*t.m.c.*) on the vegetative shoot, the other two (one

sporophylls except that the lowest two are frequently more leathery and a little smaller.

Examples were found which deviate from the usual opposite, close arrangement of the bracts. On a specimen from Java five cones are borne axillary to bracts which approach a spiral arrangement on a somewhat longer fertile branchlet. In another specimen exhibiting an approach to a spiral arrangement, although on a shorter branchlet, another small sterile bract was found near the base, several millimetres from the terminal cluster. In all clusters examined, one cone is always terminal and surrounded by sterile bracts.

Although these fertile branchlets are ordinarily borne axillary to leaves on vegetative branches normal in length, an example was found in which the vegetative branch has been considerably shortened. It bears six lateral fertile branchlets in the axils of leaf scars and one terminal cone, at the base of which are spirally arranged scars, undoubtedly those of bracts. The leaves of the shortened vegetative branch are probably reduced and scale-like.

Certainly in this species early steps in the reduction of the male fertile branch have been taken; many of the bracts and fertile branch units have been lost; those remaining have been brought into a primary cluster by reduction of internodes. The fertile branch is always terminated by a cone instead of an apical bud. Finally, the primary clusters may rarely be reduced to a single unit. A further step has been forecast, of a condition prominent in its extreme in the *Eupodocarpus* section, that of reduction in the vegetative branch which bears the fertile branches.

Several species of the *Nageia* section have been described as having 'branched cones'—namely, *P. nagi* (Thunb.) Zoll. and Moritzi, *P. nankoensis* Hayata, *P. Blumei* Endl., and *P. Wallichianus* Presl. It is clear, however, that it is not a condition of branching but rather a fusion among the units of a loose primary cluster. Since the organization of the clusters in all these species is similar except for minor details, a description of the clusters of *P. nagi* will be sufficient.

*P. nagi*, with other species of the *Nageia* section and unlike the rest of the genus, has broad leaves with many parallel veins, suggestive of species of *Agathis* in the *Araucariaceae*. Three to five male cones are borne on short

removed) lateral (*l.m.c.*) and axillary to the scale leaves. Fig. 10. *P. dactyloides*. Male fertile branch reduced to a terminal (*t.f.bra.u.*) and one lateral (*l.f.bra.u.*) fertile branch unit axillary to a bract (*brs.*). The lower part of the fertile branch bears scale leaves (*s.ls.*). Fig. 11. *P. gracilior*. Male fertile branch (*r.f.bra.*) shortened and reduced to one terminal fertile branch unit and four sterile bracts (*brs.*). Fig. 12. *P. latifolius*. Solitary male cone reduced from a secondary cluster showing outer scale leaves (*s.ls.*) of the reduced vegetative branch and large inner bracts (*brs.*) of the reduced fertile branch. Fig. 13. *P. montanus*. Portion of the vegetative cone-bearing branch (*v.bra.*) with scale leaves (*s.l.*) which subtend male fertile branches shortened and reduced to single, terminal fertile branch units (*t.f.bra.u.*) with a few basal sterile bracts (*br.*). Fig. 14. *P. amarus*. Primary cluster of male cones; the fertile branch (*f.bra.*) shortened and reduced to several lateral fertile branch units (*l.f.bra.u.*) axillary to bracts (*brs.*) and a terminal unit (*t.f.bra.u.*) with several basal sterile bracts. Fig. 15. *P. nagi*. Primary cluster of male cones showing fusion between the axes (*f.c.a.*) of two lateral fertile branch units (*c'*) and the terminal fertile branch unit (*t.f.bra.u.*). More fully explained in the text. (All figs.  $\times 4$ .)



branchlets axillary to the vegetative leaves (Fig. 15). In *P. Wallichianus* larger clusters of five, seven, or nine cones may be found. At the base of the branchlet are two opposite bracts (*br.*), broad at the base but with a long, pointed, membranous tip. At right angles to these, and 2 or 3 mm. above, there is a second pair of opposite bracts (*br.*'). The two lowest cones of the cluster (*c.*) are inserted opposite each other above the lower bracts (*br.*) and obviously belong to the axils of this pair, although their apparent insertion is even higher than the second pair of bracts (*br.*'). It is clear that the cone axes have become fused to the fertile branchlet for a few millimetres above the bract axils.

The three upper cones suggest one branched terminal cone, since sporophylls appear to be inserted on the branchlet below the apparent insertions of the two lateral cones. This is not the case, however; when the sporophylls are removed it is clear that the two lateral cones (*c.*') belong to the axils of the second pair of bracts (*br.*'), above which they occur. The 'webbing' between the terminal cone and the two lateral cones (*c.*') indicates the fusion of these three axes and may extend varying distances, sometimes as much as 4 or 5 mm. above the axils of the subtending bracts. The sporophylls cover the 'webbed' region and may be sparsely scattered a little distance below it. In one specimen examined, two more, opposite structures (*br.*"), which are interpreted as bracts, are inserted below the region of sporophylls and directly above the lower pair of cones (*c.*). These would be logically a third uppermost pair of bracts inserted at right angles to the middle pair (*br.*') and not subtending any cones, although one of the pair bears three, the other two, sporangia. The whole loose primary cluster should be interpreted as a somewhat reduced and shortened fertile branch on which the spiral arrangement of bracts has become opposite, the axes of the individual cones fused with the branch, and the upper lateral cones fused with the terminal one. No one of the individual cones may be considered 'branched'.

The dominating tendency for reduction and loss of units in the male fertile branch reaches its extreme in several species of the *Stachycarpus* group: *P. falcatus* (Thunb.) R. Br., *P. gracilior* Pilger, *P. ferrugineus* Don, and *P. montanus* Loddiges. In these species, usually single cones on short bracteate stalks are borne axillary to vegetative leaves (Fig. 11). Two or three pairs of nearly opposite coriaceous, small, obovate bracts occur consistently on the cone stalks. Usually the bract axils are sterile, but not infrequently a cone may occur axillary to one or sometimes to two of the bracts. The lateral cones are always sessile and never bear bracts on their axes. The position of the two lowest bracts is of interest and indicates that the opposite arrangement on the stalk has been derived by reduction of internodes from the spiral bract arrangement on the fertile branch of *P. spicatus*. The two lowest bracts, although they are inserted at the same level, are never directly opposite one another but are always displaced towards the side of the subtending vegetative leaf where the margins of their decurrent bases touch. These solitary cones undoubtedly represent a single, persistent, terminal fertile branch unit on an

extremely reduced fertile branch. In spite of loss of axillary units and reduction of internodes, several bracts have persisted on the short stalk.

Several examples were found in *P. gracilior* and *P. falcatus* in which a secondary cluster has been subsequently formed by extreme reduction of internodes on the vegetative branch. The terminal branch bud is usually present in the midst of the cluster. A spiral arrangement of the leaves, often reduced to scales, persists, but all the axillary primary clusters are always reduced to solitary cones. In *P. montanus* (Fig. 13) the reduced fertile branches are constantly borne axillary to vegetative leaves reduced to pointed, leathery scales (*s.l.*) on vegetative branches which have become specialized as entirely cone-bearing shoots. The branch may retain an apical bud, or a reduced fertile branch may terminate it. Ordinarily in this species, reduction in vegetative leaf size has not been accompanied by shortening of internodes. However, one example was found in which the internodes on the specialized vegetative branch have been considerably shortened, bringing the eleven solitary cones together into a secondary cluster. It is an interesting fact also that a few leaves above the bud scales on all other vegetative branches are also reduced in size, although not to the same extent as those subtending the cones.

Terminal, isolated cones occur in the *Dacrycarpus* section of the genus where the dominating tendency has been toward leaf reduction rather than branch shortening. The section includes *P. imbricatus* Blume (*P. cupressinus* R. Br.), *P. papuanus* Ridl., *P. Vieillardii* Parl., and *P. dacrydioides* A. Rich. In habit and cone position these species are particularly notable. They are all trees with richly branched crowns. Two types of leaves are borne during the life of the tree, both types often occurring on the same shoot. This character clearly shows these species to be in a transitional stage of leaf reduction. The juvenile leaves are variable in size, approximately  $\frac{1}{2}$  in. long, flat, soft, and thin, often two-ranked; the adult leaves are three-sided, imbricate, small, and appressed to the branchlets.

The small male cones (Fig. 10) are terminal and in all cases represent terminal fertile branch units. Usually all the lateral units have been lost although an occasional cone may be found in the axil of a bract (*l.f.br.u.*) close to the terminal cone. The position of the fertile branches is certainly both lateral and terminal. Fertile branches are ordinarily borne on vegetative branches with adult foliage, while leaf reduction has resulted in a strong similarity between leaves and bracts. It is, therefore, impossible to distinguish the limits between fertile branch and vegetative branch. Internodal reduction has probably occurred and some fertile branches bearing terminal cones have been extremely reduced, bringing the terminal cones down into the leaf axils in the same manner as those of *P. falcatus*, *P. ferrugineus*, and other species of the *Stachycarpus* group.

It is frequently noticeable, especially in *P. imbricatus*, that branches with juvenile leaves often bear the reduced, adult type of leaf in the basal region. The transition between the leaf types is gradual and if a terminal cone is present on such a branch, a gradual reduction in lamina again occurs in the

region beneath the cone. Bud scales, as specialized structures, are not present in this section but the reduced basal leaves here as well as those in *P. minor* and *P. vitiensis* strongly suggest the step which in other groups has led to such specialization. There are other points of similarity between the *Dacrycarpus* section and these two species. Cone position and fertile branch position are similar (Fig. 39); in both, a single, terminal fertile branch unit has been retained on comparatively unreduced fertile branches which are terminal and lateral. Clusters never occur and vegetative branching is relatively unreduced. It is conceivable that the species of this section were closely like *P. minor* and *P. vitiensis* before leaf reduction had advanced to its present stage. Although the *Dacrycarpus* section shows advanced characters in the fusion of bract to epimatium in the female and in the reduced leaf type, it nevertheless has retained primitive characters in the nature and position of its fertile branches and in the retention of a richly branched vegetative condition.

#### EFFECTS OF VEGETATIVE BRANCH REDUCTION ON POSITION OF MALE FERTILE BRANCHES

##### *Transitional clusters.*

While the *Stachycarpus* group and *Nageia* section of the genus are characterized by reduction of the primitive fertile branch to primary clusters and finally to solitary cones, the *Eupodocarpus* section possesses in general secondary clusters. The male fertile branch has likewise been reduced to the extreme of a single, terminal fertile branch unit on a bracteate pedicel varying in length and representing with the few remaining sterile bracts the vestige of the fertile branch. These reduced fertile branches, however, have been brought together into secondary clusters by reduction in the internodes of the vegetative branch on which they are borne (Fig. 39). While the secondary clusters are found only rarely in isolated cases on individual plants in the *Stachycarpus* group, they are a constant and characteristic feature in the *Eupodocarpus* section. Furthermore, the secondary clusters are reduced here in some species to single cones by a subsequent loss of reduced fertile branches from the secondary cluster. In some species it has become constant, in others both clusters and single cones occur.

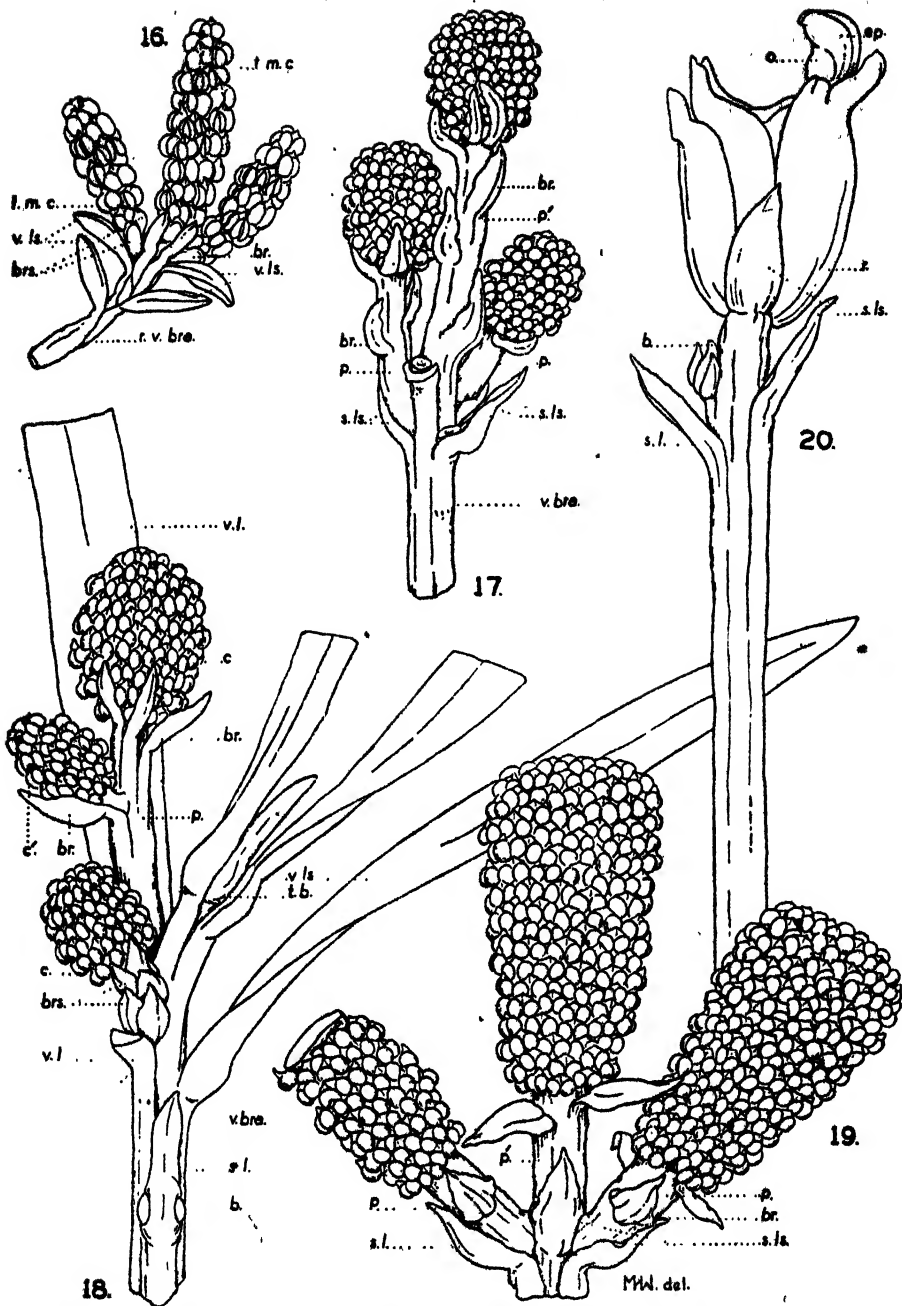
The richly branched vegetative condition of the *Stachycarpus* group and *Dacrycarpus* section is here rather rare, except in transitional species. Frequently, branching is in whorls and leaves have become opposite, often extremely crowded towards the apex of the branch. The leaf type of the section is characteristically long and linear, with a single midvein. That branch reduction is continuing within the group is shown by the fact that two or three species are frequently characterized by tertiary clusters of male cones (Fig. 39), that is, secondary clusters which have been brought together by reduction of another order of vegetative branches. In all cases transitional steps indicate the direction of reduction, frequently within one species, invariably within the section.

Two species, which clearly show the step taken towards the secondary cluster, will be considered first: *P. Drouynianus* F. Muell. and *P. alpinus* R. Br., which are probably in this respect the most primitive species in the section. They are still in the process of change and show frequent transitions even on individual plants.

The individual cones of *P. Drouynianus* (Figs. 17, 18) are always borne on a pedicel (*p.*, *p.*') on which are varying numbers (2 to 10) of spirally arranged membranous bracts. These pedicels represent the primitive fertile branch shortened and reduced to a single terminal cone. One case only was found in which a small, lateral, sessile cone (Fig. 18, *c.*') also occurred in the axil of an upper bract. These stalked cones are carried in the axils of leaves on short vegetative branches (*v.bra.*) which are in various stages of reduction. The branch may vary in length (on specimens examined) from  $2\frac{1}{2}$  cm. to a few mm. The longer branches usually bear some normal vegetative leaves and some leaves in which the laminae have been reduced nearly to scales. In other cases, all the leaves on the branch may have become scale-like. In one specimen the length of the vegetative cone-bearing branches decreases progressively from the base of the primary vegetative branch on which they are carried to its apex. In another specimen, all the vegetative cone-bearing branches have been reduced to the extreme of 2 to 3 mm., with only two decussate pairs of scale leaves remaining which surround a cluster of two or three pedicelled cones (Fig. 19). The clusters have the general appearance of being terminal on the short vegetative branches because they are usually borne in the axils of the upper leaves where the internodes are always shortened. Frequently, however, the terminal bud of the branch persists in the midst of a loose cluster (Fig. 18, *t.b.*). Otherwise, a pedicelled cone (reduced fertile branch) is in a terminal position on the vegetative branch (Fig. 10, *p.*'). Invariably such a cone has a longer pedicel and more bracts than the lateral cones of the cluster.

Branch reduction in this species, then, is in a transitional state. As a result, the reduced fertile branches may be either isolated in leaf axils on shortened vegetative branches or brought together into true secondary clusters surrounded by the scale leaves of the extremely reduced vegetative branch. All steps between these two extremes are to be found within the species.

In *P. alpinus*, another species showing transitional stages to the secondary cluster, the male cones are usually in loose clusters of three or sometimes more at the end of short branches about 5 mm. or less in length (Fig. 16). These branchlets sometimes bear a few ordinary vegetative leaves other than the crowded, terminal ones subtending cones. Ordinarily, the internodes on such branchlets are shorter and the leaves a little smaller than those on the rest of the plant. Towards the extremity of the branch the internodes are completely eliminated and four or more leaves are brought together surrounding the cluster of, usually, three cones. One cone is always in a terminal position on the branchlet; no case of a persistent apical bud was found as in the preceding species. While in *P. Drouynianus* a cone pedicel persists, it is almost eliminated



FIGS. 16-20. Fig. 16. *P. alpinus*. Secondary cluster of male cones; the reduced vegetative branch (*v.v.bra.*) bearing two lateral reduced fertile branches (*l.m.c.*) axillary to vegetative leaves (*v.la.*) and a terminal reduced fertile branch (*t.m.c.*). Several persistent sterile bracts (*bra.*) remain at the base of each individual cone. Fig. 17. *P. Drouyanianus*. Secondary cluster

in *P. alpinus*. Each individual cone axis, however, bears at its base three or four membranous bracts (*brs.*) and represents a fertile branch reduced to its single terminal unit.

In some cases the lower leaves on the vegetative cone-bearing branchlets are completely lost and the upper ones surrounding the clusters have been reduced to scales. Moreover, various stages in the shortening of the branchlet occur, from the length already described to 2 or 3 mm. With loss of lower leaves, the bases of the upper scales entirely sheathe the branchlet, now actually a peduncle. When this occurs the clusters resemble those in other species of the section that no longer show the steps leading to this reduction. This species, therefore, like the preceding one, forms an important transition to more advanced conditions prevalent in the rest of the section.

#### *Stalked and sessile secondary clusters.*

While the two preceding species are still in the process of change, the following group have attained peduncled secondary clusters of male cones as a fixed character. The group includes *P. nivalis* Hook., *P. acutifolius* Kirk, *P. Totarra* Don, *P. Hallii* Kirk (in the last species the secondary clusters are more usually reduced to single cones, but unlike most other single cones in the section, the peduncle, a remnant of the vegetative branch, persists), *P. Parlatoarei* Pilger, *P. Lambertii* Klotsch, *P. glomeratus* Don, and *P. elongatus* (Ait.) L'Hér.<sup>1</sup> A description of the clusters in *P. nivalis* and some unusual specimens of *P. Totarra* will suffice to illustrate the effects of extreme reduction in the vegetative branches.

In *P. nivalis* (Fig. 9) there is a cluster of generally three cones at the end of a common peduncle 6 to 12 mm. in length. The clusters occur axillary to

<sup>1</sup> This African species has been frequently confused in vegetative characters with *P. falcatus* (Stapf, 1933, p. 8). Specimens in several herbaria, even those bearing male cones, were erroneously labelled *P. falcatus*. The latter species, in the *Stachycarpus* group, has primary clusters of male cones; *P. elongatus* has secondary clusters. Solitary cones occur in both species, but the single cones of the *Stachycarpus* group lack scale leaves outside the bracts, which are always present in solitary cones which have been reduced from the secondary clusters of the *Eupodocarpus* section. Compare, for example, Figs. 11 and 12.

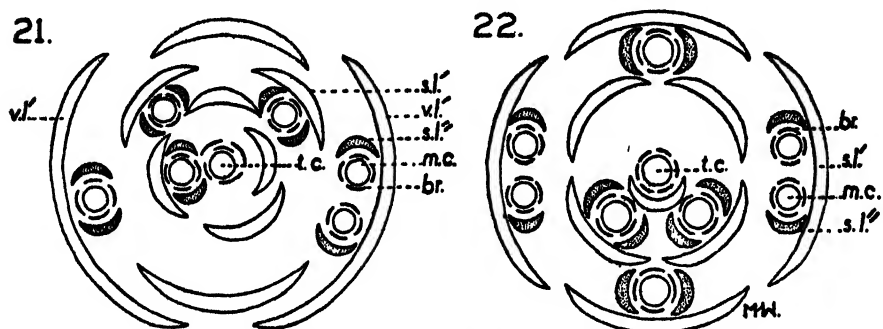
of five male cones (two removed). The shortened vegetative branch (*v.bra.*) bears scale leaves (*s.ls.*) in the axils of which are borne the fertile branches, each reduced to a solitary, terminal fertile branch unit on a bracteate pedicel (*p.*). One reduced fertile branch (*p.*') is terminal on the short vegetative branch. (Compare with Fig. 18.) Fig. 18. *P. Drouynianus*. A short vegetative branch (*v.bra.*) with a terminal bud (*t.b.*) bears some normal vegetative leaves (*v.ls.*) and some leaves reduced to scales (*s.l.*). One axillary fertile branch has been reduced to a bracteate pedicel (*p.*) with one terminal fertile branch unit (*c.*) and one persistent lateral fertile branch unit (*c.*') axillary to one of the bracts (*br.*). A lateral bud (*b.*) is in the axil of a scale leaf (*s.l.*) of the vegetative branch. Fig. 19. *P. Drouynianus*. A sessile secondary cluster of male cones, surrounded by persistent scale leaves (*s.ls.*) of the greatly reduced vegetative branch. The terminal (*p.*') and two lateral (*p.*) pedicels each represent a reduced fertile branch with one persistent terminal fertile branch unit and several sterile bracts (*br.*). Fig. 20. *P. Drouynianus*. Greatly reduced female fertile branch, the bracts of which are specialized in forming with the axis a fleshy receptacle (*r.*). Axillary to one bract is a young ovule (*o.*) only partially covered by the developing epimatium (*ep.*). The receptacle is borne on a long peduncle which seems to represent a reduced vegetative branch with several scale leaves (*s.ls.*), one of which subtends an axillary bud (*b.*).

vegetative leaves. The peduncle (*r.v.bra.*) is sheathed by the decurrent bases of two pairs of thick, fleshy, reduced leaves (*s.l.*) which surround the cluster. The lower pair are larger and often approach in size and form the ordinary vegetative leaves. Otherwise they are more reduced in size, as are the opposite upper pair, which are inserted at right angles to the lower. The basal margins of the lower scales meet on the side towards the subtending vegetative leaf and frequently one member of the pair is inserted a little lower than the other. The two lateral cones (*l.m.c.*) are axillary to the two lower scale leaves, which represent with the upper pair the only leaves retained on a much shortened vegetative branch (*r.v.bra.*). Rarely the lateral cones are lost from the cluster and a single one, terminal on the short vegetative branch, persists. At the base of each individual cone axis, inside the surrounding scale leaves, are three to five thin, membranous bracts (*brs.*), the greater number on the terminal cone. Each individual cone represents a fertile branch reduced to one terminal fertile branch unit and several sterile bracts.

The male cones of *P. Totarra* are more usually borne singly, but may be, as in the preceding species, in clusters of two or three on a common peduncle varying in length from 2 or 3 to about 5 mm. The single cones or clusters are surrounded by two pairs of leathery scale leaves, the decurrent bases of which sheathe the peduncle. Within the outer reduced leaves, each individual cone axis bears at the base two to four membranous bracts in opposite pairs. Occasionally a few more than the usual four scale leaves are present; in such specimens a close spiral arrangement persists. In a few instances, not all the leaves are reduced to scales; they resemble the normal foliage leaves but are only half their size. Frequently a terminal cone with one lateral cone is present.

An interesting branch was found in material collected by Professor Eames in New Zealand. The apex of the branch (which is about 6 in. in length) has been injured and four or five lateral branchlets have been produced just below the injured portion. The lower part of the branch bears normal peduncled cones axillary to leaves, some solitary, others in twos. On the lowest of the new distal branchlets are two lateral cones, in the axils of the first two opposite bud scales. The branchlet is therefore in a median position between them; instead of producing a terminal cone (reduced fertile branch) it has continued vegetative growth. The four opposite bud scales at the base are identical in appearance and arrangement with the four scale leaves surrounding the normal clusters, except that the branchlet with its two lateral cones is sessile. The individual cones, on the other hand, unlike those of the normal clusters, are pedicelled, each representing a less reduced fertile branch with its persistent terminal fertile branch unit. On one cone pedicel there are eight bracts—one low and isolated, the next two opposite, and the others spirally arranged. On the other pedicel one bract is also low and isolated; two more are opposite, but inserted close beneath the sporophylls. Such abnormalities clearly indicate the steps by which reduction has taken place and like the two transitional species are in this respect of particular interest.

In another group of species with male cones organized similarly to the preceding group, the peduncled secondary clusters of three or four cones have been reduced to a sessile condition by further reduction in the vestigial vegetative branch (Fig. 19). The cluster is surrounded by two decussate pairs of thick, leathery scale leaves set close together. As in the peduncled clusters the



FIGS. 21-2. Diagrams of male cone arrangement in tertiary clusters. Fig. 21. In *P. salignus*. Fig. 22. In an undetermined Podocarpus. *br.*, bract; *m.c.*, male cone; *s.l.*', scale leaf on reduced vegetative branch of the first order; *s.l.*', scale leaf on vestigial vegetative branch of the second order. (These scale leaves more often occur as two decussate pairs surrounding a secondary cluster or single cone reduced from a secondary cluster. Only a single pair has been shown in each case in order to simplify the diagram.) *t.c.*, terminal male cone; *v.l.*', unreduced vegetative leaf on reduced vegetative branch of the first order.

outer two are displaced towards the side of the subtending vegetative leaf. These scale leaves are all that remain of the vegetative branch. The short individual cone pedicels always bear several pairs of small membranous bracts, the larger number on the central cone, which would represent the reduced fertile branch which had been terminal on the disappearing vegetative branch. To this group belong *P. elatus* R. Br., *P. novae-caledoniae* Vieill., *P. macrophyllus* (Thunb.) Don, *P. polystachys* R. Br., *P. neriifolius* Don, and *P. milanjanus* Rendle. Unlike the former group, most of which were limited to high altitudes, this group, with the exception of *P. milanjanus*, is typical of low altitudes and tropical areas.

#### *Solitary cones reduced from secondary clusters.*

The following group generally bear solitary cones: *P. latifolius* (Thunb.) R. Br., *P. Brassii* Pilger, *P. Nakaii* Hayata, *P. glaucus* Foxworthy, *P. angustifolius* Griseb., *P. coriaceus* Rich., and *P. Matudei* Lundell. The solitary male cones (Fig. 12) are sessile in the axils of vegetative leaves. At the base of each cone are two pairs of opposite, leathery scale leaves (*s.l.s.*) narrower and often more pointed than the inner bracts. The outer pair of scale leaves are inserted at the same level but are displaced towards the side of the subtending vegetative leaf where their basal margins touch. The inner bracts (*brs.*) on the short cone pedicel are closely arranged in nearly opposite pairs, often tending towards a spiral. They are usually membranous, broad, and rounded, except in



*P. Brassii*, in which the very numerous bracts are long and pointed, more nearly resembling the outer scale leaves. In number these bracts may vary from six to nine pairs; in *P. Brassii* they are the most numerous. That these solitary cones are reduced from secondary clusters is evident from their organization and from the fact that such single cones are frequently found in species in which the secondary clusters are the more usual condition.

### *Tertiary clusters.*

Two species of the *Eupodocarpus* section that were examined showed steps in the formation of tertiary clusters, namely, *P. salignus* Don and a specimen which could not be determined with certainty.<sup>1</sup> The male cones of *P. salignus* are borne in loose tertiary clusters, formed by the shortening of the primary vegetative branches, of which the terminal bud may persist or may be replaced by a terminal cone. The arrangement of these tertiary clusters can best be understood from the diagram, Fig. 21. The lower part of the branchlets often bears three or four leaves (*v.l.*) in whose axils are usually single cones or sometimes a secondary cluster of two or three. These secondary clusters or single cones have the usual two pairs of opposite scale leaves (*s.l.*) surrounding them and the individual axis of each cone (*m.c.*) bears at its base two to four opposite bracts (*br.*). The lower leaves on the branchlet may be separated by short internodes, or in some cases two may be attached at the same level but be displaced from an opposite position, so that the margins of their decurrent bases touch at one side. There is always a long internode between these leaves and the terminal cluster. At the apex of the branchlet, the internodes of the leaves (*s.l.*) have been lost, bringing the axillary cones together into a group. This tertiary cluster may vary in four particulars. (1) The subtending leaves of the branchlet may all be the size of ordinary vegetative leaves; some may be reduced; or all may be reduced to scale-like proportions. (2) These same leaves may vary in number and in position from spiral to opposite. (3) Either a terminal cone (reduced from a secondary cluster) or a terminal branch bud may be present. (4) The number of cones in the apical cluster may vary considerably. The secondary clusters may be composed of two, very rarely more, cones or frequently they may be reduced to a single unit.

Advance in reduction is found also in the sporophylls of this species. The lamina is completely lost and the stalk so minute that the two spherical sporangia have the superficial appearance of being attached directly on the cone axis over which the sporophylls are loosely scattered.

The male cones of the undetermined species are extremely advanced in their arrangement (Fig. 22). They are grouped in large tertiary clusters of three to nine cones, borne on naked peduncles 10 to 15 mm. long. At the apex of the peduncle, and surrounding the entire cluster, are two decussate pairs of narrow,

<sup>1</sup> It is labelled *P. nubigenus* Lindl. and was collected in Cochabamba, Bolivia. In comparison with specimens of *P. nubigenus*, however, it proved not to be that species, nor could it be identified as either of the two species listed from Bolivia, *P. oleifolius* and *P. Parlatoresi*. It bears the closest resemblance perhaps to *P. glomeratus*, which is recorded only from Peru and Ecuador.

long-pointed, reduced leaves (*s.l.*) with decurrent bases extending the length of the peduncle. In one cluster of nine cones examined, the two outer reduced leaves each bear an axillary, secondary cluster of two cones. Each secondary cluster is again enclosed at the base by two pairs of opposite scale leaves (*s.l.*), which represent the remnants of a vegetative branch of the second order. Within these leaves each individual cone possesses the usual three or four basal bracts (*br.*). Only single cones instead of secondary clusters are present in the axils of the inner pair of scale leaves. Of the three remaining cones, one is terminal (*t.c.*) and the other two in adjacent axils of an inner whorl of four more scale leaves. A ninth leaf is inserted against the terminal cone. Each single cone, axillary to the scale leaves of the branchlet, is surrounded by two to four more scale leaves outside its bracts, indicating it to be a single cone remaining from a secondary cluster. The relations are clearer in this species than in the preceding one, because some differences still exist between the reduced leaves of the two orders of vegetative branches and the bracts. The scale leaves of the branchlet (of the first order) are distinguishable by their long-pointed woody tips; the scale leaves of the second order, surrounding the secondary clusters, have acuminate apices which are shorter or nearly lacking; while the bracts are rounded and membranous.

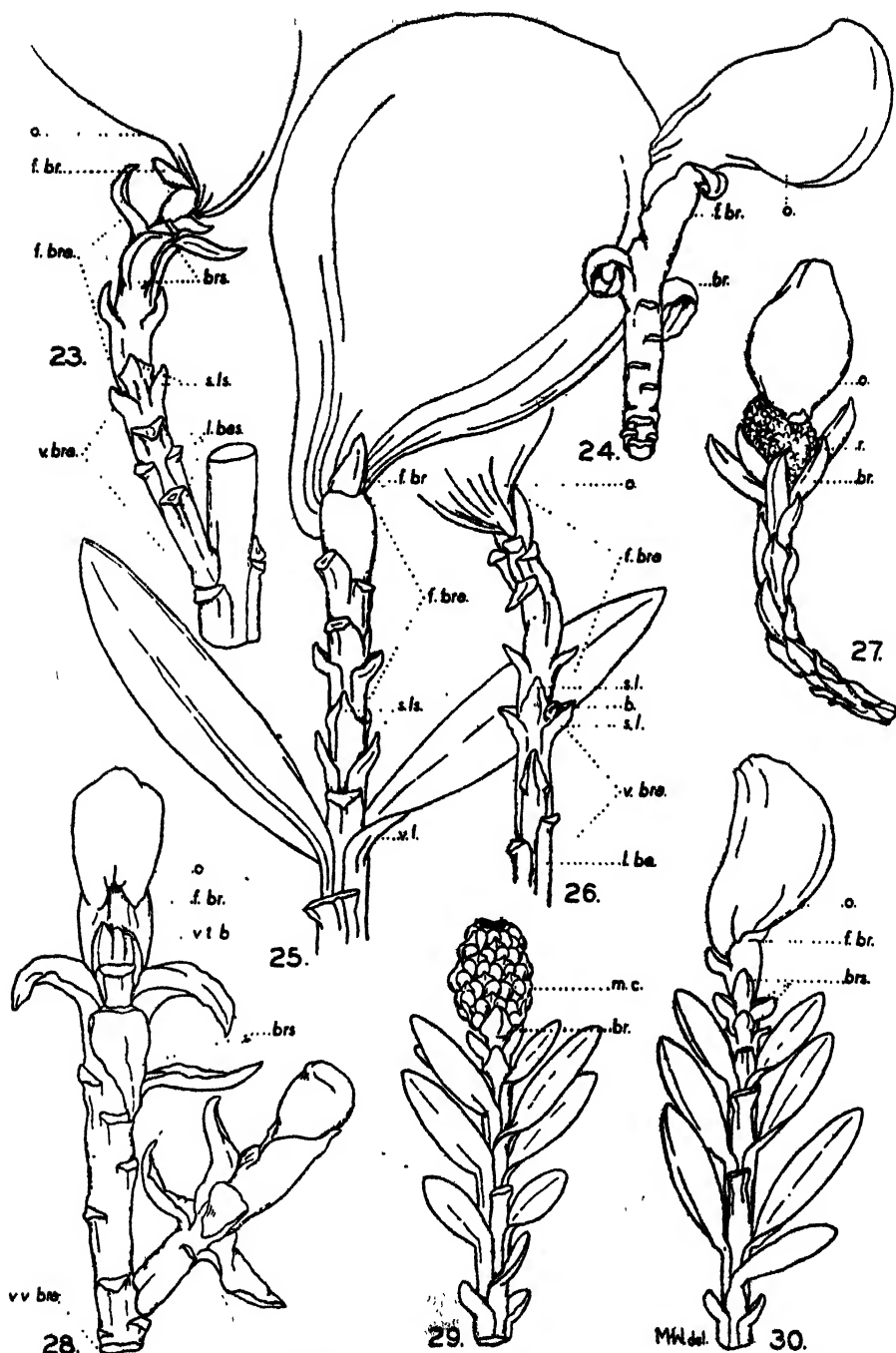
It is to be suspected that advanced stages of tertiary clusters exist in other species. This may be a more common condition in the *Eupodocarpus* section than is now evident. Even some single cones surrounded by an unusually large number of foliar structures, as those of *P. Brassii*, are possibly derived by loss of cones from tertiary rather than secondary clusters.

#### EVOLUTION OF THE FEMALE FERTILE BRANCH

##### *The primitive condition.*

The female fertile branches of *P. spicatus*, similar in length to, or sometimes shorter than, the male, bear small, spirally arranged bracts (Figs. 1, 8, E). Approximately eight of the upper bracts are fertile; in the axil of each is a single ovule enclosed in its epimatium (Fig. 2). As already mentioned, these axillary units of the female fertile branch are greatly reduced secondary shoots and are thus homologous with the small, unreduced fertile branch units of the male—the staminate cones.

In comparing the male and female fertile branches of this species, the only difference occurs in the more restricted region of fertility in the female. Only approximately eight of the upper bracts bear fertile axillary units, in contrast to the twenty or thirty frequently fertile in the male. The lower sterile bracts (or scale leaves) are thinner, more membranous, progressively closer together, and clustered at the base as bud scales. In herbarium material it is difficult to find many specimens in which the lower bracts have persisted. Pilger (1926, p. 220) again cites instances in which fertile branches, occurring terminally on vegetative branches, produce somewhat leafy bracts at the base of the fertile



FIGS. 23-30. Fig. 23. *P. vitiensis*. Female fertile branch (*f. bra.*) bearing sterile bracts (*brs.*) and one persistent fertile branch unit, the ovule (*o.*), enclosed in an epimedium in the axil of a fertile bract (*f. bra.*). The fertile branch is borne terminally on a shortened vegetative branch (*v. bra.*) on which are leaf bases (*l. bas.*) and terminal scale leaves (*s. l.*). ( $\times 4$ .) Fig. 24. *P. gracilior*. Female fertile branch reduced to one fertile branch unit, the ovule (*o.*) enclosed in an

branch, which almost reach the foliage leaves in size. Gibbs (1912, p. 539), who observed this species growing in New Zealand, writes: 'In every case the axis was terminated by a bud pushed on one side by the growth of the last fertile bract. In no case, and a great deal of material was available, were the bud scales and modified leaves of the axis and the apical bud absent.' In one instance only, however, does she record these sterile bracts or scale leaves to have the size of normal vegetative leaves. And this may have been due to the fact that the terminal bud had been destroyed, causing the lower bracts to grow out to the size of leaves.

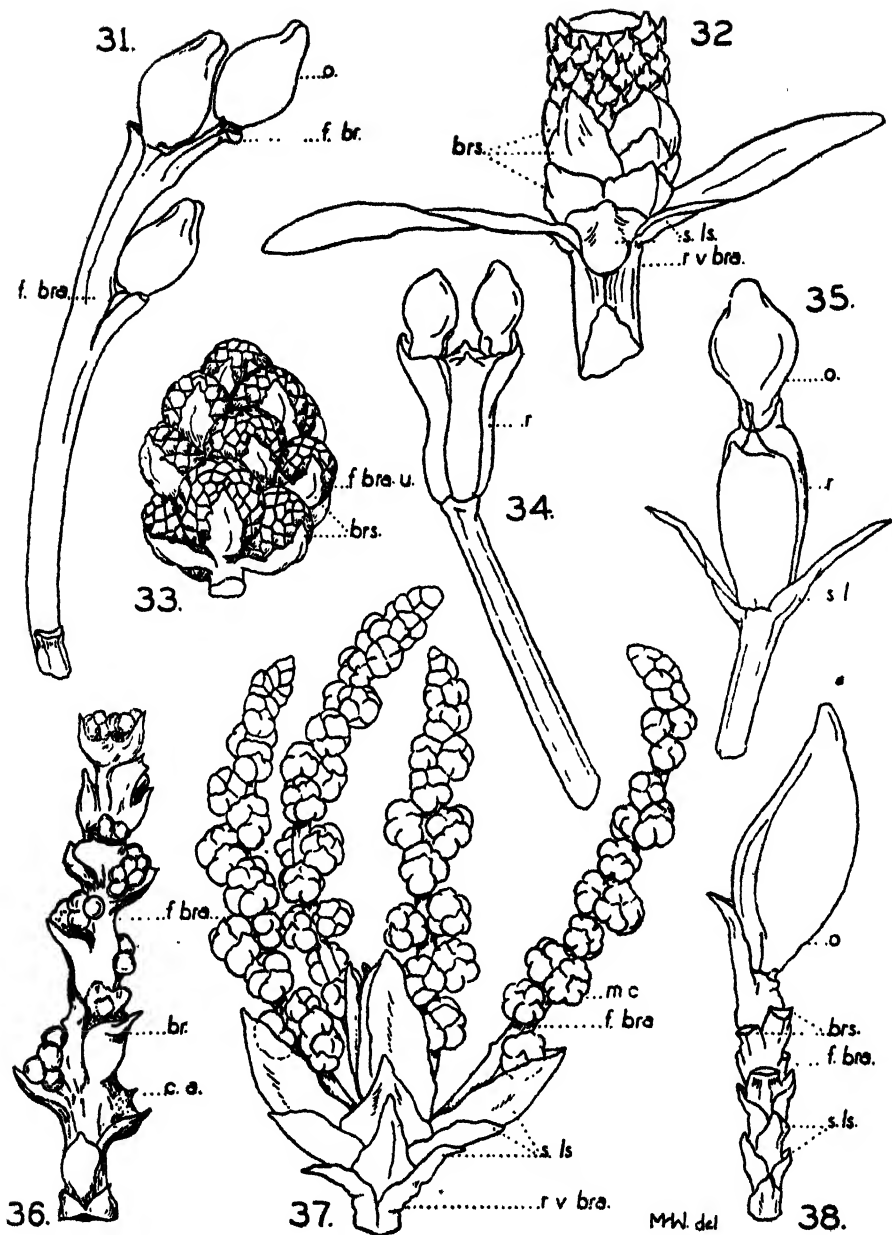
The organization of the female fertile branch in *P. andinus* (Fig. 6) is essentially similar to that in *P. spicatus*. In none of the limited number of specimens examined is there any increase in the size of the sterile bracts at the base of the fertile branches. In fact, those above the bud scales are dry and scaly and more closely similar to the bud scales than to the fertile bracts. Pilger (1926, p. 220) observed that two conditions may occur in this species: the whole branchlet may be fertile or only the upper part. When the fertile branch occurs in a terminal position he says that the 'modified leaves' at the base of the fertile branch almost equal the foliage leaves in size and the limits of the branch are uncertain. In all the material I examined, however, the fertile branch was limited by the presence of bud scales at its base.

### Reduction of units.

*P. amarus* is the only other species constantly to retain two or three fertile branch units. These units occur near the apex of the fertile branch, separated by short internodes (Fig. 31). The basal region is usually bare and shows the scars of only two or three sterile bracts, separated by wide internodes from the distal bracts. The condition is comparable to that of the male of this species in which a loose primary cluster of two to five cones is borne at the end of a short branchlet.

As in the male fertile branches, reduction occurs in number of fertile branch units to a single terminal or subterminal one. This is accompanied in the male by extreme shortening of the fertile branch; in the female, however, the fertile branch retains not only nearly its original length, but frequently a

epimatium, and axillary to a fertile bract (*f.br.*). ( $\times 4$ .) Figs. 25-6. *P. vitiensis*. Female fertile branches (*f.bra.*) each bearing a solitary persistent fertile branch unit, the mature ovule (*o.*) enclosed in an epimatium, and axillary to a fertile bract (*f.br.*). The fertile branch in each case is borne terminally on a normal vegetative branch (*v.bra.*), the apical leaves of which have been reduced to scales (*s.ls.*) with shortened internodes. In Fig. 26 a lateral bud (*b.*) occurs in the axil of a scale leaf. ( $\times 4$ .) Fig. 27. *P. Vieillardii*. Female fertile branch bearing proximal scale leaves and distal sterile bracts (*br.*) some of which have formed with the branch axis a fleshy, warty receptacle (*r.*). A solitary terminal fertile branch unit, the ovule enclosed in an epimatium, has persisted. The fertile bract which is not visible is fused with the epimatium. ( $\times 4$ .) Fig. 28. *P. Blumei*. Two female fertile branches, one lateral, the other terminal, borne on a vestigial vegetative branch (*v.v.bra.*). One fertile branch has retained a vestigial apical bud (*v.i.b.*). ( $\times 4$ .) Fig. 29. *P. minor*. Male fertile branch bearing proximal leaves, distal bracts (*br.*), and a solitary, terminal fertile branch unit or male cone (*m.c.*). ( $\times 1\frac{1}{2}$ .) Fig. 30. *P. minor*. Female fertile branch bearing proximal leaves, distal bracts (*brs.*), and a solitary terminal fertile branch unit, the ovule (*o.*) enclosed in an epimatium. ( $\times 1\frac{1}{2}$ .)



FIGS. 31-8. Fig. 31. *P. amarus*. Female fertile branch (f.br.), bearing three fertile branch units (o.) axillary to bracts (f.br.). ( $\times 2$ .) Fig. 32. *Agathis robusta*. Solitary male cone reduced from a secondary cluster. On the reduced vegetative branch (r.v.bra.) are two decussate pairs of scale leaves (s.ls.). The cone bears at its base several large bracts (brs.) and represents the single, terminal fertile branch unit of a greatly reduced fertile branch. ( $\times 2$ .) Fig. 33. *Sciadopitys verticillata*. Young male fertile branch bearing numerous fertile branch units (f.br.u.) or male cones axillary to bracts (brs.). ( $\times 2$ .) Fig. 34. *P. salignus*. Two fertile branch units, young ovules each enclosed in an epimatium, seated on a receptacle (r.) which is formed by the swollen bract bases fused with the axis of the fertile branch. The long naked peduncle

marked differentiation into a distal fertile portion and a proximal vegetative one. In *P. montanus*,<sup>1</sup> *P. Mannii*,<sup>1</sup> and *P. minor* (Fig. 30) the proximal vegetative portion of the branch bears normal unreduced vegetative leaves with four to six bracts in the distal region under a terminal ovule. These female fertile branches resemble the male fertile branches of *P. minor* (Fig. 29) and *P. vitiensis* except for the presence of bud scales in *P. montanus* and *P. Mannii*. In *P. falcatus* and *P. gracilior* (Fig. 24) the basal leaves are reduced to early deciduous scales while the three or four upper bracts are persistent. In *P. ferrugineus* (Fig. 38) the basal scale leaves (*s.l.*) are numerous, small, and imbricating and pass gradually into the larger bracts (*brs.*) which are, on the contrary, early deciduous, with the exception of the fertile one.

In the *Nageia* section *P. nagi* bears on young fertile branches two ovules more often than one, each in the axil of opposite bracts at the apex of the branch. Between them is invariably the tip of the branch with two undeveloped bracts on either side. Four to six fleshy, sub-opposite bracts are still persistent beneath the ovule. On the lower part of mature branches are four to six scars, the decurrent bases of these deciduous scale leaves are still prominent. Several species of the *Nageia* section show a tendency for the bracts to become swollen and fleshy—a condition which is doubtless the forerunner of the receptacle which is a constant character of the *Eupodocarpus* section. As Pilger points out, however, this receptacle in species of the *Nageia* section differs from that in the *Eupodocarpus* section in that the stalk is not naked, but carries scale leaves, and the receptacle is formed from a larger number of bracts in a spiral rather than an opposite arrangement.

The female fertile branches in the *Dacrycarpus* section (Fig. 27) resemble those of the male. In all four species a single terminal fertile branch unit has been retained, the lateral ones lost. Although in the male it is impossible to distinguish between the bracts of the fertile branch and the reduced leaves, in the female the bracts of the upper fertile portion of the branch are larger, while in the basal sterile region the leaves are reduced to imbricating scales. A few of the upper bracts directly below the ovule are fused into a fleshy,

<sup>1</sup> Unfortunately no female specimens of these two species could be obtained. Excellent illustrations, however, of *P. montanus*, under the synonym *P. taxifolius* Kunth., have been published (Richard, 1826, Pl. 29; Humboldt and Bonpland, 1817, t. 97). Pilger (1926, p. 244) has illustrated *P. Mannii*, which is a rare, endemic species from the Island of St. Thomas.

seems to represent a reduced vegetative branch on which the solitary fertile branch is terminal. (× 4.) Fig. 35. *P. neriifolius*. Similar to Fig. 34, except that only one ovule has persisted and the reduced vegetative branch bears two scale leaves (*s.l.*). Fig. 36. *Austrotaxus spicata* (A drawing kindly sent me by Professor R. B. Thomson of Toronto). Male fertile branch (*f.bra.*) bearing small reduced cones axillary to bracts (*br.*). The reduced cone axis (*c.a.*) is fused with the subtending bract. (× 4.) Fig. 37. *Amentotaxus argotaenia*. A secondary cluster of male fertile branches (*f.bra.*). The shortened vegetative branch (*r.v.bra.*) bears several pairs of opposite scale leaves (*s.l.s.*) surrounding the cluster. A terminal bud is present at the centre. The small male cones borne on the fertile branches have lost their subtending bracts. (× 2.) Fig. 38. *P. ferrugineus*. Female fertile branch (*f.bra.*) with one terminal fertile branch unit, the ovule (*o.*) surrounded by an epimatium. The proximal part of the branch bears scale leaves (*s.l.s.*), the distal part the bases of deciduous bracts (*brs.*). (× 4.)

warty receptacle (*r.*) at maturity; the bract which subtends the ovule is fused with the epimatium which it equals in length.

#### EFFECTS OF VEGETATIVE BRANCH REDUCTION ON POSITION OF FEMALE FERTILE BRANCHES

##### *Clustering of female fertile branches.*

Evidence of reduction in the vegetative branches is to be found in its early stages in *P. falcatus*. A specimen was found, comparable to a frequent type in the male, in which the female fertile branches are borne on a reduced vegetative branch. In this specimen three female fertile branches are carried in a terminal cluster on a short vegetative branchlet which has retained its terminal bud in the midst of the cluster. This condition is also figured by Stapf (1933, p. 11). In *P. Blumei* the reduction has evidently proceeded further. In this species I found an example of what has been described for *P. vitiensis* as a 'branching peduncle'. A lateral fertile branch bears in the axil of one of its lowest scale leaves a second shorter fertile branchlet (Fig. 28). The longer branch bears distally a single ovule (*o.*), a vestigial terminal bud (*v.t.b.*), and eight persistent bracts (*brs.*) in opposite pairs. Below these are six scars sub-oppositely arranged; at the base, two very nearly opposite scars, one of which subtends the shorter fertile branch. This branchlet bears one terminal ovule and six persistent close bracts in opposite pairs, with two opposite scars directly under the bracts. Below the scars the branchlet is naked to the base, a distance of one-third of its length.

The question now arises as to whether the branching is actually in the fertile branch. I think, however, there is another, more plausible, interpretation. Blume (1847, Tab. 173),<sup>1</sup> in a beautiful illustration of this species, shows a vegetative branch on which three female fertile branches are clustered at the apex. One fertile branch is terminal on the vegetative shoot, the two lateral fertile branches are inserted close to the terminal one, making a cluster of three. If such a vegetative branch were reduced in length, the cluster would be brought down into the leaf axil of the primary vegetative branch. Furthermore, the distinguishing limits between the reduced vegetative shoot and the terminal fertile branch would gradually be lost and vegetative leaves would be reduced to scales. The so-called 'branching peduncle', therefore, may represent rather a branching in a vestigial portion of the vegetative shoot at the base of the terminal fertile branch (Fig. 28, *v.v.bra.*).

Further evidence is to be found in *P. vitiensis* (Fig. 25). Under a terminal ovule the fertile branch (*f.bra.*) bears six to eight bracts arranged in sub-opposite pairs, one of which subtends the ovule. The lower part of the branchlet is covered with imbricating scale leaves, probably reduced from a basal vegetative region such as that in the male fertile branch. Gibbs (1912, p. 534) describes the character already mentioned for *P. Blumei*: 'The strobili are borne on branching peduncles which arise on the axils of the lower leaves of

<sup>1</sup> Figured under the synonym *P. agathifolia* Bl.

the shoot in great profusion.' Her term 'peduncle' refers to the lower portion of the fertile branch which is covered with scale leaves. None of my specimens of *P. vitiensis* shows branching such as Gibbs describes. Evidence, however, from the material examined shows that the interpretation of the 'branching peduncles' of this species should be similar to that of the same condition in *P. Blumei*. Three of the five female fertile branches, on the one specimen I was able to examine, are clearly terminal on short vegetative branches (Figs. 25, 26) instead of lateral as Gibbs describes them. At the apex of these short vegetative shoots what appear to be the last two pairs of leaves have been reduced to scales (*s.l.s.*) and the internodes between them nearly lost. The scale leaves at the base of the fertile branch are above these and, higher up, separated by longer internodes. In one instance, the scar of what was probably another fertile branch (broken off) shows in the axil of one of these terminal scale leaves of the vegetative shoot and in another a bud (Fig. 26, *b.*) is present. This is comparable to what has been described for *P. Blumei*, three clustered fertile branches at the apex of a short vegetative shoot. In this case one fertile branch is terminal and the limits between vegetative and fertile branch already indistinct because of the reduction in lamina of the vegetative leaves. As in *P. Blumei*, with continued shortening of such a vegetative shoot the truly terminal fertile branch would finally appear lateral on the primary vegetative branch. The vestige of vegetative shoot left at its base would still retain the power of branching and produce a lateral as well as a terminal fertile branch. Moreover, elsewhere on the same specimen two superficially lateral fertile branches both bear evidence of such a vestigial vegetative branch at the base of the fertile branch (Fig. 23). Above scars (*l.b.s.*) of what appear to be six vegetative leaves (possibly reduced) two pairs of persistent scale leaves (*s.l.s.*) are inserted close together, with internodes shortened as they are at the apex of the unreduced cone-bearing vegetative branches. That truly lateral fertile branches, however, do occur is shown by Gibbs's illustrations of some lateral fertile branches which lack these basal vestiges. Such an interpretation of the 'branching peduncles' is reasonable in the light of the obviously strong tendency for branch reduction throughout the whole genus.

The first effects of vegetative branch reduction have been shown to result in clustering; a subsequent tendency, already visible in *P. vitiensis* and *P. Blumei*, is a reduction in the number of lateral fertile branches formed. As will be seen in the *Eupodocarpus* section, this tendency results finally in the complete elimination of truly lateral fertile branches and the retention only of those in a terminal position on the shrinking vegetative branch.

#### *Reduction in number of female fertile branches.*

The presence of a fleshy receptacle, formed by the swollen bract bases fused with the fertile branch, is a constant and distinguishing character of the *Eupodocarpus* section. A single ovule, rarely two, is retained in most of the species. The receptacle, immediately beneath the ovule, is generally formed of the two



upper opposite bracts, between which the tip of the branchlet and two more or less undeveloped bracts is sometimes evident (Figs. 34, 35). Frequently at maturity the receptacle (*r.*) becomes a brilliant red or purple. The laminae of the bracts are greatly reduced and are sometimes evident only as small points on the mature receptacle. The receptacle is always borne on a short naked stalk, varying in length from a few mm. to 1 or 2 cm. At the top of this stalk, and immediately below the receptacle, in two-thirds of the species of the group, occur two opposite, narrow, dry or leathery little scale leaves, whose long decurrent bases sheathe the peduncle (Fig. 35, *s.l.*). These are often shed when the ovule reaches maturity. They are lacking in the other third of the species (Fig. 34). In some, however, the stalk may show a slight bulge at its apex, with traces of the decurrent bases. Pilger (1926, p. 222) notes that the presence or absence of these little leaves at the base of the receptacle corresponds among the species with certain geographical limits. Those species in which they are present are often tropical forms. The species which have apparently lost these two bracts are those frequently of high altitudes or temperate climates.

The nature of this naked peduncle and its two little scale leaves is not entirely clear. Pilger (1926, p. 224) believes the scale leaves to be of the same nature as the upper bracts which form part of the receptacle, because occasionally in *P. spinulosus* and *P. Drouynianus* they may also become fleshy and take part in the formation of the receptacle. The naked stalk, then, with its two scale leaves would correspond to the basal sterile portion of the fertile branch. This, of course, is a logical and very probable interpretation. Other evidence, however, should be taken into consideration. The reduction of male fertile branches in the *Eupodocarpus* section has been accompanied by reduction in vegetative branches both of the first and second order. This reduction is also noticeable in the general habit of these trees in comparison with the richly branched forms of the *Stachycarpus* and *Dacrycarpus* groups. It is to be suspected, therefore, that such vegetative branch reduction, which is clearly obvious in its relation to the position of male fertile branches, has been correlated also with the nature and position of the female fertile branches. Although such correlation seems reasonable to expect, it may not necessarily have occurred, since all the species of this section are dioecious. Transitional steps, so plentiful in the male, are unfortunately generally lacking.

Some evidence on this point was found, however, in one species, *P. Drouynianus*, certainly with respect to male fertile branches the least reduced member of the section. Specimens were found in which other leaves, reduced and normal, also occur on a longer peduncle, as well as the usual condition in which two opposite scale leaves are directly below the receptacle. On one such specimen a small aborted shoot is visible above the axil of one member of an extra pair of subopposite scale leaves near the apex of the peduncle (Fig. 20, *b.*). It consists of two tiny bracts with what looks like the rudiments of an aborted ovule between them. This suggests the 'branching peduncles' of the *Nageia* section and such forms as *P. falcatus* and *P. vitiensis* in which both terminal

and lateral fertile branches are borne at the ends of reduced vegetative shoots.

Another interesting deviation was found in this species. Below the receptacle, the lamina of one small leaf is also fleshy; an opposite one is nearly the size of a normal vegetative leaf. It is thin and green, with the exception of one small spot at the base of the lamina which has become fleshy. A few millimetres lower on the stalk another normal-sized leaf is present, and still lower, two more, subopposite scale leaves, not reduced to the usual extreme. The whole stalk measures 2.7 cm. in length, somewhat longer than the usual peduncles.

The foregoing evidence suggests the fact that possibly this usually naked peduncle represents a reduced vegetative branch on which the fertile branch is terminal (Fig. 40). The lower sterile portion of the fertile branch, having been merged into the vegetative branch so long and so completely, is either lost entirely or its limits are unrecognizable. If this peduncle represents only the basal sterile portion of the fertile branch, one would not expect to find the rudiments of a second fertile branch in the axil of one of its scale leaves. Moreover, just such a naked peduncle, bearing four scale leaves at its apex around a secondary cluster of cones, is the form which the reduced vegetative branch has assumed in the male. Here one reduced fertile branch is usually terminal on the peduncle, the others lateral. In the female, if the latter interpretation be valid, only the terminal fertile branch has been retained, the lateral ones lost.

## DISCUSSION

### *Fertile branches and relationships in Podocarpus.*

The entire evolution of the male fertile branch in the Podocarpaceae has been preserved within the genus *Podocarpus*; this genus contains representatives of the most advanced as well as the most primitive types. The *Stachycarpus* group contains those species which exhibit the fertile branch, both male and female, in its most primitive form, as well as those which show its first modifications. Moreover, a primitively richly branched habit is prevalent. Branch reduction in this group has involved only the fertile branches, normally not the vegetative; nor has specialization in the development of a female receptacle occurred. The male fertile branch has progressed in reduction through loose primary clusters ultimately to a single fertile branch unit (Fig. 39). The female fertile branch, without the extreme internodal shortening that has characterized the male, has likewise been reduced in its number of fertile branch units to a single one (Fig. 40). The group is, therefore, a natural one and forms the most primitive division of the genus.

The subgenus *Protopodocarpus* is not as natural, nor as clear-cut a division, since it unites in its sections species which differ in very fundamental characters. Pilger (1926, p. 242) defines it as: 'female flowers axillary or rarely

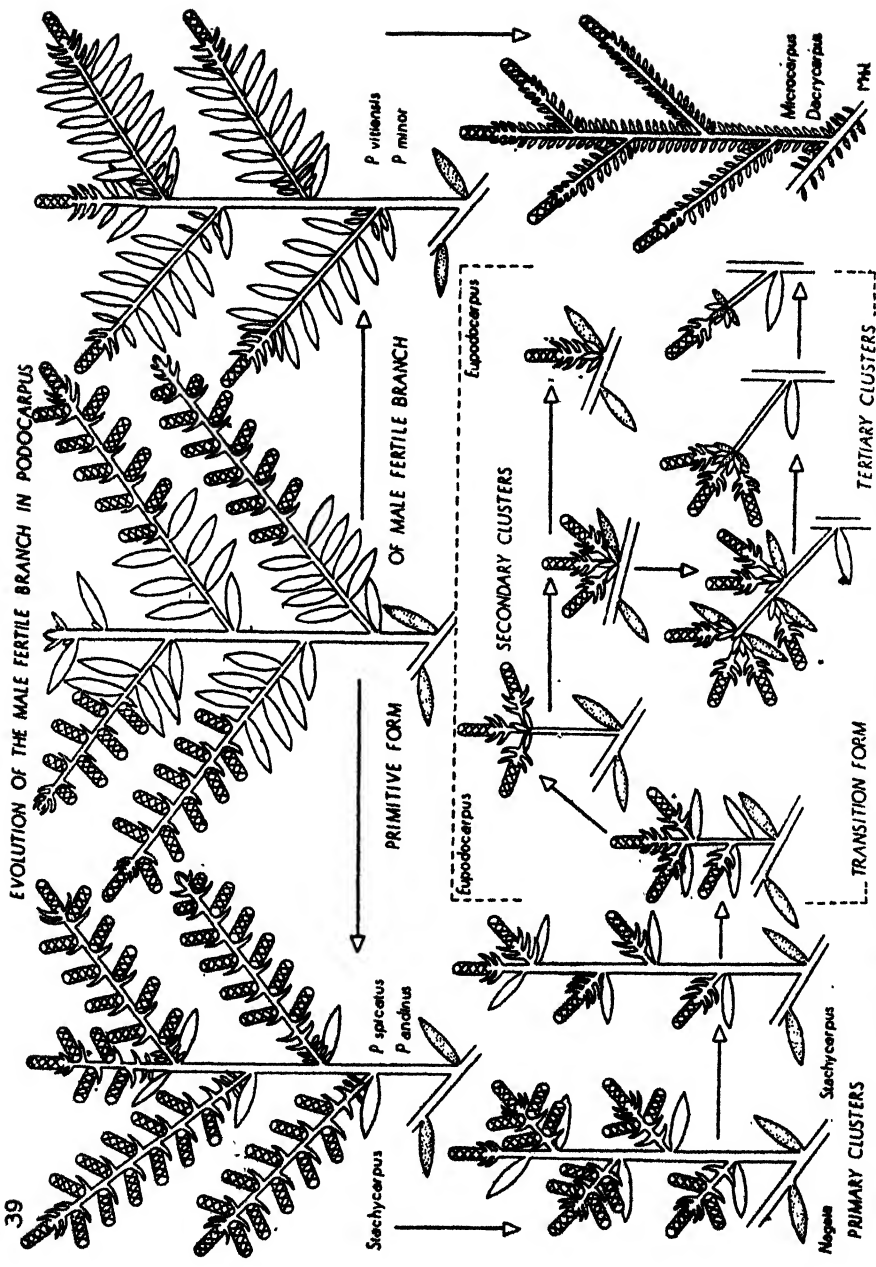


FIG. 39. Diagram showing the evolution of the male fertile branch in *Podocarpus*. Explanation in text.

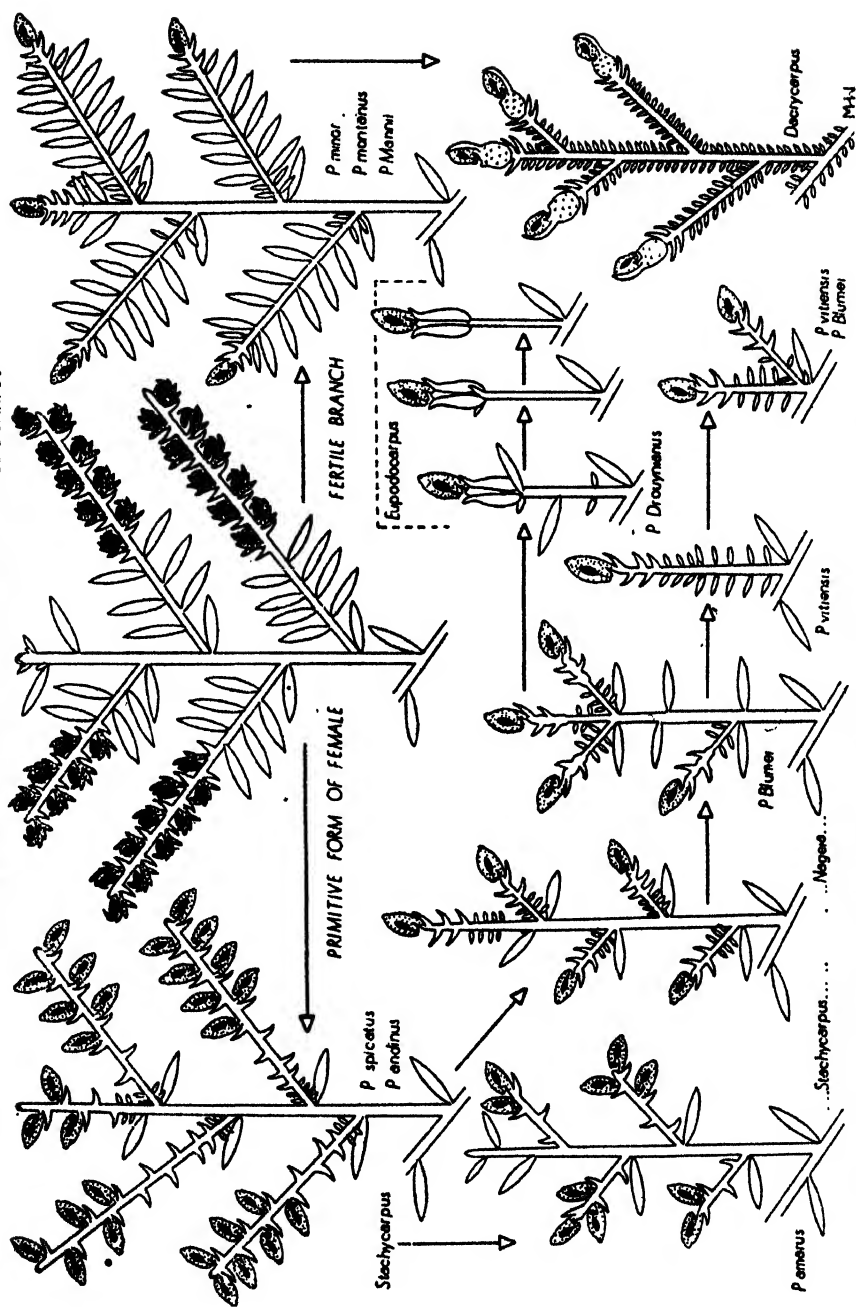


FIG. 40. Diagram showing the evolution of the female fertile branch in Podocarpus. Explanation in text.

terminal on branchlets; receptacle mostly developed.' Within the group he unites the many-veined *Nageia* species, the extremely specialized and reduced *Eupodocarpus*, the richly branched, small-leaved *Dacrycarpus* species, and *Microcarpus*, with only a single representative.

The *Nageia* section certainly stands apart in its large, many-veined leaves, closely similar to those of *Agathis* and some of the *Cordaiales*. Species of this section are not particularly richly branched and the leaves are opposite to sub-opposite. Their male fertile branches are entirely in loose primary clusters. That some vegetative branch reduction has occurred is indicated by the clustering of the female fertile branches in some of the species. However, it is not unlikely that in both the *Nageia* section and the *Cordaiales* rich branching is not a primitive character, but that primitive aggregates of telomes were united to form the many-veined leaves. In other species of the genus, the richly branched condition may be associated with single-veined leaves, not reduced from a many-veined condition, but primitively single-veined in their origin. Although the male fertile branch of *Nageia* is reduced to a primary cluster of cones, the female fertile branch has generally lost all but a single, terminal unit. These fertile branches are like those of the *Stachycarpus* group and bear no resemblance to the secondary and tertiary clusters of *Eupodocarpus* or the isolated branches of *Dacrycarpus*. Nor is the thickening of the bract bases of the female fertile branch in some species similar to the receptacle of *Eupodocarpus* on its naked stalk. Several species, moreover, are monoecious. Since there are no other characters to unite the *Nageia* section with either *Eupodocarpus* or *Dacrycarpus*, it would seem better to raise it to the rank of a subgenus, parallel with *Stachycarpus*.

The *Eupodocarpus* section of the genus shows strong evidence of vegetative branch reduction and, as a result, the aggregation of reduced male fertile branches into secondary and tertiary clusters (Fig. 39). It is to be suspected, as already discussed, that the stalk and scale leaves below the receptacle in the female represent a reduced vegetative branch on which the female fertile branches, also once clustered, are now reduced to a solitary, terminal one (Fig. 40). The arrangement of fertile branches is, therefore, in this section particularly distinguishing. They bear no similarity in position to those of the *Dacrycarpus* section in which clustering never occurs. The sections are similar in the possession of a receptacle and in the reduction of the individual fertile branches to one single, terminal fertile branch unit. The latter character, however, is not sufficient to unite these sections into one subgenus, when the position of the fertile branches is so dissimilar.

The *Dacrycarpus* section, like some species of the *Stachycarpus* group, is characterized by similar male and female fertile branches. A single, terminal fertile branch unit is a constant character, and fertile branches are lateral and terminal (Figs. 39, 40). The species are exceedingly richly branched; reduction has, rather, affected leaf size. The unreduced juvenile leaves, moreover, are more suggestive of the *Stachycarpus*-*Taxus* type than of the long linear leaves of most of the *Eupodocarpus* species. The receptacle is rough and

warty in contrast to a smooth condition in the *Eupodocarpus* species, and the laminae of the bracts, less reduced, project from the fleshy fused bases. The fertile bract in this section has become fused to the epimatium.

The *Microcarpus* section, with its single species, *P. ustus*, is likewise characterized by a richly branched habit and reduced leaves. Fertile branches are apparently similar to those of *Dacrycarpus*. There is no fusion of bract to epimatium, and Pilger describes the receptacle as little developed.

A consideration of the two uncertain species of the *Nageia* section, *P. minor* and *P. vitiensis*, may now be undertaken. It has already been mentioned that they exhibit in their fertile branches primitive features found elsewhere only in the female fertile branches of *P. montanus* and *P. Mannii*, which differ from them in the possession of basal bud scales. Gibbs (1912), after an anatomical investigation of *P. vitiensis*, found evidence for excluding it from the *Stachycarpus* group and agreed with Pilger on the advisability of leaving it provisionally in the *Nageia* section. Unlike species of *Nageia*, however, both *P. minor* and *P. vitiensis* have single-veined leaves, a character alone certainly sufficient to exclude them from that section. The possibility may be considered, however, that these two species are more closely allied to the *Dacrycarpus* section. Like species of *Dacrycarpus* their fertile branches are isolated, only slightly shortened in length, but reduced to a single terminal unit (Figs. 39, 40). With the exception of *P. vitiensis* in which vegetative branch reduction has begun, vegetative branches are comparatively unreduced, a richly branched habit has persisted, and bud scales are lacking. *P. vitiensis* and *P. minor* may represent the remnant of forms from which the *Dacrycarpus* section has been derived. *P. ustus* may represent a transition in that its fertile bract is still free from the epimatium and receptacle development is slight. The steps to the *Dacrycarpus* section would consist in a uniform internodal and leaf reduction, reduction in the size of the ovule with fusion to the fertile bract, and finally the development of a receptacle.

If, then, both the *Nageia* and *Eupodocarpus* sections were given subgeneric rank parallel with *Stachycarpus*, the subgenus *Protopodocarpus* could consist of a new section for *P. minor* and *P. vitiensis*, the *Microcarpus* section, and the *Dacrycarpus* section. These would be united on the basis of fertile branch nature and position, and would represent a more natural grouping. The two species in question, however, should first be more thoroughly investigated.

The evolution of the male fertile branch in *Podocarpus* may be traced in Fig. 39, the evolution of the female fertile branch in Fig. 40. The arrows indicate merely the probable sequence in reduction and are not indicative of phylogenetic relationships.

#### *Nature of the primitive fertile branch.*

In the nature of their compound fertile branches, *P. spicatus* and *P. andinus* are probably the most primitive living conifers. Like the *Cordaitales*, they possess similar male and female fertile branches, specialized in bearing determinate, secondary, axillary shoots on which are aggregated ultimate fertile

appendages. In both the male and female a sterile portion exists at the base of the branch, including the bud-scale region. The distal fertile portion in the female shows evidence of reduction from the more extensive fertile region in the male. Was the branch primitively entirely fertile? Although a terminal bud has been found to be constantly present at the apex of the female fertile branch, in the male a terminal fertile branch unit may occur. Was the primitive fertile branch originally determinate or indeterminate? The bracts often resemble the foliage leaves in form if not in size. Is the bract a reduced vegetative leaf or a structure primitively always associated with the secondary fertile shoot and never vegetative in function?

The possibility certainly exists, in the light of the progressive sterilization of the fertile branches in *Podocarpus*, that they were primitively entirely fertile. Such branches may have been both determinate and indeterminate, since both types occur in these two species. Another possibility is that the fertile branches were primitively differentiated into a basal vegetative portion, bearing normal vegetative leaves, and a distal fertile portion. The occurrence of such a basal vegetative portion in the male and female fertile branches in *P. minor*, in the male branches of *P. vitiensis*, and in the female branches of *P. montanus* and *P. Mannii* is evidence that such a type must have existed. In other species of the *Stachycarpus* and *Nageia* group, a differentiated basal region is present on the female fertile branches. The foliar structures are reduced, usually deciduous, and differ from the upper bracts of the fertile region which are longer persistent. The female fertile branches of *Saxegothaea* and *Acropyle* likewise show a differentiated basal region. Furthermore, in the *Pinaceae* young female cones of *Pseudotsuga*, *Tsuga*, *Keteleeria*, *Pinus*, and other genera have short bracteate peduncles which evidently correspond with that region.

The primitive fertile branch would also, like that of *P. minor* and *P. vitiensis*, lack bud scales. It is clear from the present study that the presence of bud scales in the *Coniferae* is associated with branch reduction and elimination. Besides these two species, bud scales are lacking in the family in richly branched species where internodal shortening, which may have occurred to some extent, was of uniform nature and all leaves were reduced to scales.

It is also probable that the fertile branches were primitively indeterminate, that the determinate condition, in which a fertile branch unit is formed terminally, arose with the beginning of the tendency toward reduction. It became most strongly expressed in the male, where elimination of units and shortening of internodes has occurred in every family of the conifers. The persisting male cone is always the terminal unit of the fertile branch (Fig. 8, G). Traces of the indeterminate condition persisted, however, in the female. In *P. spicatus*, Gibbs (1912) states that the terminal bud is never absent. The terminal branch bud or a remnant of it occurs frequently elsewhere in the *Podocarpaceae*, while the proliferating cone axes often reported in *Larix*, *Cryptomeria*, *Cunninghamia*, *Araucaria*, and other genera are probably the persistent expression of the primitive indeterminate nature of the branch.

Wills (1910, p. 288-91) has also observed proliferated female cone axes in numerous fossils of *Voltzia heterophylla* Br., a Palaeozoic conifer. In position, the fertile branches are formed both laterally and terminally on vegetative branches, but we have no evidence at the present time that a completely indeterminate vegetative branch condition is more primitive.

In considering the nature of the bracts we also have little evidence. They sometimes approach the foliage leaves in size as well as form and by Pilger (1926) were considered to be reduced vegetative leaves. It is also possible, however, that the bract has always been associated with the fertile secondary shoots from its origin, never possessing the large photosynthetic lamina of vegetative leaves. Evidence of its primitive form exists in the Palaeozoic conifers, in which Florin (1939, p. 560) describes two genera, *Lebachia pini-formis* and *Walchia germanica* with bracts of the female cone bifurcated at the apex. In several species, especially in the *Nageia* section, the bracts may also become fertile, in some cases bearing more sporangia than the sporophylls.

A final consideration may be given to the fertile branch units or secondary fertile shoots. We have no evidence that these shoots were ever other than determinate. Florin (1939) so interprets them in the Cordaitales. They represent axes upon which the ultimate appendages or primitive telomes both fertile and sterile have been aggregated. Their reduction in the female is no longer a matter of speculation. Florin's investigation on the Palaeozoic Cordaitales and Coniferae has given the fossil confirmation which we so needed to substantiate anatomical, developmental, and teratological evidence.

The clue to the nature of the simple male cone arises from Florin's recognition of the compound nature of both male and female fertile branches in the Cordaitales, and their similarity to female fructifications in the ancient Coniferae. If such a similarity existed in the female, it is logical to expect such a similarity also to have occurred in the male. The relationship between Cordaitales and Coniferae, through whatever family, has long been recognized on other grounds. No fossil evidence has yet been found to show that compound male as well as female fertile branches occurred in the Coniferae. Surprisingly, such evidence exists in a living family.

We have now a clear homology for the male cone in the reduced, secondary axillary structures of the female cone—the seed-scale complex (Fig. 8, Fa, Ha). It follows, therefore, that the old homology (Chamberlain, 1935, p. 292) of the male sporophyll with the bract of the female cone can no longer stand. The male sporophyll is homologous with the vestigial appendage bearing the ovule, which with the sterile appendages makes up the ovuliferous scale. Although the male secondary fertile shoots of *Cordaianthus Penjoni* were described as bearing both sterile and fertile appendages, the male sporophylls of our present-day coniferous cones are all fertile with the occasional exception of one or two at the base. In the primitive female secondary fertile shoot, likewise, both sterile and fertile appendages were present. It is possible that the entirely fertile condition is more primitive or that sterile appendages have been incorporated into the formation of the present-day sporophyll, much as



the ovuliferous scale has fused with the ovular stalk. The problem undoubtedly needs further investigation in the light of new homologies.

*Modification of the primitive fertile branch.*

As already stated, the most primitive known condition of the fertile branches occurs in *P. spicatus* and *P. andinus*. Outside the Podocarpaceae a few genera in other families have retained male fertile branches with numerous fertile branch units, generally modified in some other way. In the Taxodiaceae, *Sciadopitys verticillata* (Thunb.) Sieb. and Zucc. possesses a male fertile branch (Fig. 33) much like an ordinary female cone of *Pinus*. Each fertile branch unit or male cone is sessile and axillary to a membranous bract (*br.*). Internodal shortening between units has resulted in a compact, ovoid structure, actually a compound male cone in the same sense as the female is a compound cone.

In the Taxaceae, *Austrotaxus spicata* Compton still possesses fertile branches probably unreduced in number of units, although greatly modified (Fig. 36). The original interpretation of these fertile branches, first stated by Compton (1922, p. 427) in his original description of the plant, later by Doyle (1926, p. 160), and finally by Saxton (1934, p. 421) is inaccurate in the light of the present investigation. They have described the branch as a strobilus bearing sporophylls in the axils of bracts. Pilger (1926, p. 211), however, interprets it as: 'Male flowers [cones] in little spikes in the axils of leaves or scale leaves; spikes with 12 to 15 little, wide bracts; flowers with 1 to 5 shield-shaped stamens with short filament; sporangia 2 to 4.' I could obtain no material of *Austrotaxus* to examine, but I am indebted to Professor R. B. Thomson of Toronto for confirmation of my belief that these spikes represent male fertile branches. He has examined anatomically some of Compton's original material and has kindly permitted me to use his drawing of the fertile branch (Fig. 36). That the groups of sporophylls axillary to the bracts (*br.*) are actually borne on much reduced secondary axes (*c.a.*) is evident from the vascular anatomy. Professor Thomson writes: 'The two bundles for the cone come off laterally to the bract bundle and these divide into five for the sporophylls, two of which (the basal) bear two sporangia each and the other three one each.' In his recent article (1940, p. 75) he also mentions the fact that 'the cone is fused throughout its entire length with the subtending bract. . . .' This is an interesting condition and one which parallels the reduction of the secondary fertile shoot in female cones, with its final fusion to the subtending bract in the Araucariaceae and Taxodiaceae.

*Amentotaxus argotaenia* (Hance) Pilger, in the Cephalotaxaceae, has also retained modified male fertile branches probably unreduced in number of units (Fig. 37). In the limited amount of material I could examine the male fertile branches (*f.bra.*) bear small, sessile, spherical cones (*m.c.*), but subtending bracts are entirely absent, although Pilger (1926, p. 270) describes the cones as axillary to very small bracts. The bracts, therefore, instead of the fertile branch units are apparently disappearing. Again I am indebted to

Professor Thomson for anatomical evidence. He writes: 'In *Amentotaxus* the bract bundle seems to go directly to one sporophyll in some cases at least, and so would seem to represent a greater amount of fusion than in *Austrotaxus*.' In these three genera, therefore, outside the Podocarpaceae, the compound male fertile branch has been preserved.

#### *Position of the fertile branches.*

Two definite lines of reduction are to be recognized, not only within Podocarpus but within the Coniferae as a whole. The primitive conifer was undoubtedly a richly branched plant with unreduced leaves and abundant fertile branches. Whatever the cause, inhibitions of growth became manifest in one of two ways. Either the richly branched condition was retained and leaf lamina extremely reduced, or branch reduction and elimination occurred with relatively little change in leaf. The latter has been the predominating tendency in all the Coniferae and directly the cause of the great variety of male and female cone arrangement.

The Stachycarpus group, together with *P. minor* and *P. vitiensis*, are in habit probably the most primitive, in that they have retained both rich branching and relatively unreduced leaf, although evidence of branch reduction in its earliest stages is already apparent. Besides the Dacrycarpus section of Podocarpus, Dacrydium, Microcachrys, and Pherosphaera in the Podocarpaceae, the Eutacta section of Araucaria, and probably the Cupressaceae with some genera of the Taxodiaceae are representatives of leaf-reduction types. In such forms the male fertile branches remain abundant and unclustered. In the Podocarpaceae they are generally little reduced in length, produced laterally and terminally, and characterized by reduction of units generally to a single terminal one. There is no interruption in the leaf, bract, and sporophyll series. In the Cupressaceae, however, cone pedicels are naked, showing that reduction has occurred in a different manner. Although that manner is not readily apparent, it is hoped that a later study of the family will reveal the steps.

There is a strong similarity to the Podocarpaceae in the nature and arrangement of the fertile branches in Araucaria. The Eutacta section of the genus bears solitary terminal male cones. It is evident that, like the above-mentioned genera of the Podocarpaceae, the cone represents a terminal fertile branch unit on a long fertile branch from which the lateral units have been lost. Reduction has affected leaf size rather than branching, with the result that a superficial similarity exists between the vegetative leaves, bracts, and sporophylls. This similarity has been emphasized as a primitive character by those who draw homology between male and female cones, but it is evident that, far from being a primitive character, it occurs only as a result of foliar reduction.

That some distinction still exists between the fertile and vegetative branches is shown by a statement of Thibout (1896, p. 94) that in the Eutacta section the fertile branches have leaves relatively large and thin, while on the sterile

branches they are straight and thick. Toward the apex of the fertile branch, he says, the leaves are gradually reduced and transformed into little brown scales, 'floral bracts', by reason of their position at the base of the 'flowers'. The bracts in their turn pass by degrees into the stamens. In the *Colymbia* section of the genus, the fertile branches have been reduced in length, bringing the cones down into an axillary position. It is also an interesting fact that all the fossil genera of Coniferae described by Florin (1929, 1938, 1939), as *Lebachia*, *Ernestiodendron*, *Walchia*, and *Ullmania* were richly branched forms with isolated terminal male and female cones (Fig. 8, D).

The results of vegetative branch reduction, which accompany modification of the male fertile branch, are more complicated. It has been shown that the simple male cone as it is found to-day in most of the Coniferae, in particular in *Podocarpus*, is a single terminal surviving unit with several persistent bracts of a fertile branch, once compound like the female (Fig. 8, G). The many subsequent arrangements of these single remaining male cones is brought about entirely by the degree of vegetative branch reduction characteristic of the genus or family.

The male cones of *Agathis* in the *Araucariaceae* show evidence that they are single cones remaining from secondary clusters similar to those in the *Eupodocarpus* section of *Podocarpus*. The male cones of *Agathis robusta* (Moore) F. Müll. offer the clearest evidence (Fig. 32). They are single and axillary, surrounded at the base by two decussate pairs of reduced leaves (*s.l.s.*) whose bases sheathe a short peduncle. The outer pair, in the material examined, are approximately  $2\frac{1}{2}$  cm. long, 5 mm. in width, and displaced toward the side of the subtending vegetative leaf, where their basal margins nearly touch. The inner pair are short, ovate, and the size of the bracts on the pedicel. Within these outer scale leaves are ten or twelve rounded, ovate, convex bracts (*brs.*), considerably larger than the sporophylls to which they bear no resemblance. These outer scales represent the vestige of a reduced vegetative branch upon which the fertile branches were once borne.

In the *Pinaceae*, clusters of staminate cones in *Keteleeria* and *Pseudolarix* are large, flat-topped umbels, surrounded by numerous scale leaves at the apex of more or less shortened shoots. Sometimes, moreover, trace of a terminal bud is present in the midst. The inner cones of the cluster have lost their subtending scale leaves, except for an occasional survival. Since these clusters and also the single cones of *Cedrus*, *Larix*, and *Pinus* differ from those in the *Podocarpaceae* in various morphological details, it is necessary to reserve certain interpretation of them until the family can be studied more thoroughly.

In the *Cephalotaxaceae* vegetative branch reduction has preceded fertile branch shortening and loss of units in the male, with the result that clusters of male fertile branches are characteristic of *Amentotaxus* (Fig. 37), with a somewhat more reduced umbel in *Cephalotaxus*. The male fertile branches in *Austrotaxus* in the *Taxaceae* are axillary to vegetative leaves and still not clustered. In the *Taxodiaceae* *Cunninghamia lanceolata* (Lamb.) Hook.

possesses intercalary clusters, apparently secondary. The region in which the cones are borne is compact and enlarged, with internodes shortened and leaves reduced to scales. In a young condition the cluster appears terminal; but a terminal vegetative bud in the centre of the cluster later develops.

The position, likewise, of the female fertile branches is affected by branch shortening. With progressive reduction in the secondary vegetative branches, a few female fertile branches become segregated at the apex. Lateral ones are lost, but a single terminal one persists. These steps are to be followed through the *Stachycarpus* and *Nageia* groups (Fig. 40). Although there is reduction in fertile branch units in both the male and female fertile branches of the Podocarpaceae, in other families the female fertile branches have been organized into cones without loss of units (Fig. 8, C, F). As in the Podocarpaceae, however, reduction in number of fertile branches is associated with vegetative branch shortening.

Finally, the effect of branch reduction on leaf arrangement is well known. In the Podocarpaceae, in the *Eupodocarpus* section, leaves become crowded at the apex of the shoots, and branches are whorled. In other families may be mentioned the clustered leaves of *Larix*, *Cedrus*, and *Pseudolarix*; the fused double leaf of *Sciadopitys*, and the fascicles of *Pinus*. All these, as well as the fertile branches, have been affected by the dominating tendency in the group. Frequently branch shortening in its ultimate stages becomes associated with reduction of surviving leaves to scales. It is also evident from the present study that the so-called bud scales surrounding the male cones are morphologically surviving bracts and scale leaves, still persistent from disappearing branches. The individual cones of the unreduced primary fertile branches of *P. spicatus* and *P. andinus* are sessile and have no trace of any bracts at their bases. With reduction of the fertile branch, the one terminal surviving cone is then borne on a bracteate pedicel which, with its bracts, is the persisting remnant of the shortened fertile branch. With secondary clustering on disappearing vegetative shoots, the surviving leaves, reduced to scales, surround the clusters which may finally lose all but a single cone.

A preliminary survey of other conifer families shows that all have been affected by the dominant tendency for branch or leaf reduction which is the direct cause of the apparent dissimilarity in the organization of the reproductive structures. Evidence is sufficient for us to assume that the primitive type of both male and female fertile branch was probably the same for every family. In this sense the conifers are monophyletic and have probably arisen from a common homosporous stock. How many lines evolved from this primitive stock is still problematical. Parallel development has undoubtedly occurred, in habit, in evolution of the seed-scale complex, in the simple male cone, and finally in branch reduction. The danger lies in mistaking parallel development as indicative of a closer relationship. Until a thorough and comparative study of fertile branch reduction and arrangement in all the families of *Coniferae* has been completed, phylogenetic conclusions cannot be evaluated.

## SUMMARY

1. Extensive investigation by Rudolf Florin on reproductive structures of the Palaeozoic Cordaitales and Coniferae has established two important facts: (a) that the Cordaitales possessed similar compound male and female fertile branches, composed of numerous secondary, determinate fertile shoots axillary to bracts; (b) that the ovuliferous scale of the conifers with its ovules is reduced from a determinate, secondary axillary shoot and its spirally arranged sterile and fertile appendages.

2. A morphological study of male cones in *Podocarpus* has shown that two species, *P. spicatus* and *P. andinus*, also possess similar compound male and female fertile branches. The female fertile branches bear spirally arranged bracts; in the axil of each is a single ovule surrounded by an epimatium (ovuliferous scale), which represents, in reduced form, a secondary, determinate fertile shoot and its appendages. The male fertile branch likewise bears spirally arranged bracts; in the axil of each is a small, sessile male cone, a relatively unreduced secondary, determinate fertile shoot. Both male and female fertile branches, therefore, are composed of homologous secondary fertile branch units.

3. Modifications of the male fertile branch within the genus consists of the following steps: (a) internodal reduction forming primary clusters of cones; (b) continued internodal reduction with loss of all fertile branch units except the single terminal one which has a short stalk and retains only four to six persistent sterile bracts.

4. Subsequent internodal shortening of the secondary vegetative branch brings the axillary male fertile branches (reduced to a single terminal unit) together into secondary clusters. Ultimately these clusters may be reduced to a single cone.

5. Internodal reduction in the primary vegetative branch may also bring secondary clusters of male cones together into tertiary clusters.

6. Species in which marked vegetative branch reduction has not occurred are characterized by foliar reduction. Fertile branches are isolated and little reduced in length, but have suffered reduction of lateral fertile branch units, ultimately to a single terminal one.

7. A preliminary survey of male cone arrangement in other families of Coniferae has shown that in these also a similar reduction has occurred, with some differences in morphological detail.

8. The Araucariaceae closely resemble *Podocarpus* in position and organization of their fertile branches.

9. It may be concluded that primitive Coniferae possessed a richly branched habit with similar compound male and female fertile branches; that the simple male cone, a single surviving unit of the primitively compound male fertile branch, is thus homologous with the seed-scale complex, the reduced axillary secondary unit of the female cone; that the great diversity in position of male cones is due to subsequent vegetative branch reduction.

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# A Critical Examination of New Theories of the Metabolism of Major Nutritive Elements in Plants

BY

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With three Figures in the Text

AS the result of a series of investigations carried out at the Cotton Research Station, Trinidad, Mason and Phillis have developed new theories relating to certain physiological aspects of plant nutrition. These are far-reaching in scope, and before they can be accepted a close examination of the foundations on which they rest seems desirable. This paper aims at such a scrutiny, and covers both the experimental procedure adopted and the statistical methods employed. The first part deals with the theory of apolar adsorption, the second with factors determining water content of leaves.

*Apolar adsorption.* In a series of experiments Phillis and Mason (1942) undertook to identify the factors controlling protein regulation in the leaf; analysis of their data led them to novel views regarding the partitioning of nitrogen into protein and crystalloid fractions, of phosphorus into soluble and insoluble fractions, and of 'labile carbohydrate' into polysaccharides and soluble sugars. Cotton was grown in water and sand culture under a wide range of conditions, thus obtaining an 'overlapping chain of data from very low to very high nitrogen levels'. The nutrient variants included nitrogen, phosphorus, and potassium supply. Leaves, sometimes grouped according to height on the stem, were subsequently analysed for total-N and crystalloid-N. Results are presented in graphical form only, and in Fig. 1 is shown the relation obtained between protein-N and crystalloid-N contents. The following claims are made: (1) the points from the various experiments merge into one curve; (2) a well-defined maximum value for protein-N content occurs in the middle of the range of crystalloid-N; and (3) these same results, presented as a correlation diagram (Fig. 2) between 'partition index' (i.e. percentage of total-N appearing in the insoluble form as protein-N) and crystalloid-N content, can be represented extremely closely by the straight regression line drawn, the correlation coefficient being  $-0.994$ . The hyperbola in Fig. 1 has been calculated from the regression line of Fig. 2.

Similar, though less regular, data are presented for insoluble-P and soluble-P contents (Phillis and Mason, 1942, Fig. 5), but here the experimental data lie along the falling limb of the hyperbola only; the rising limb is obtained by extrapolation and is unsupported by any data. Again the 'partition index' is



highly correlated with soluble-P content ( $r = -0.963$ ). Similarly with contents of polysaccharide and soluble sugar (Fig. 3).

On the basis of these results it is claimed that in each case the equilibrium between insoluble and soluble fractions is determined almost exclusively by

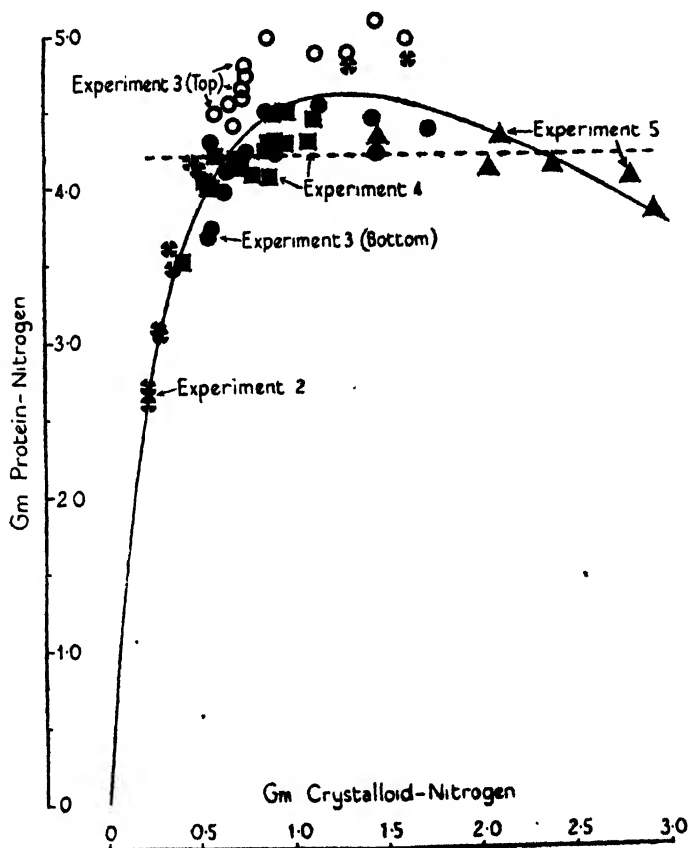


FIG. 1. Relationship between protein-N and crystalloid-N contents, reproduced from Phillis and Mason (1942). The curve was calculated from the straight regression line in Fig. 2. The inserted broken line represents a constant protein-N content (4.2 per cent.).

the actual concentration of the soluble fraction itself, e.g. the partitioning between protein-N and crystalloid-N at equilibrium depends solely on the internal level of crystalloid-N. If this simple conclusion were well founded it would dismiss as irrelevant most of the previous work on nitrogen metabolism; therefore it requires critical examination. The postulated relationship is grounded on the hypothesis that before conversion into protein the crystalloid-N must be adsorbed; in apolar adsorption, if the quantity of substance adsorbed be plotted against the equilibrium concentration per unit volume of the adsorbed substance, a curve somewhat like that of Fig. 1 is frequently

obtained. Apolar adsorption, it is held, will similarly account for the partition of both phosphorus and carbohydrate.

At this point some general questions may be raised. It appears doubtful whether the 'equilibrium concentration per unit volume' is at all adequately represented by crystalloid-N content. Without in any way questioning the important role which adsorption may play it is legitimate to inquire of what, on the hypothesis put forward, the adsorbing surface consists—presumably not the protein itself nor any surface quantitatively dependent upon protein,

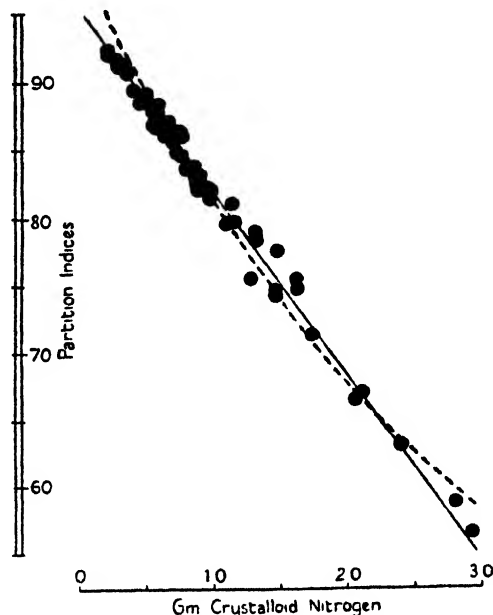
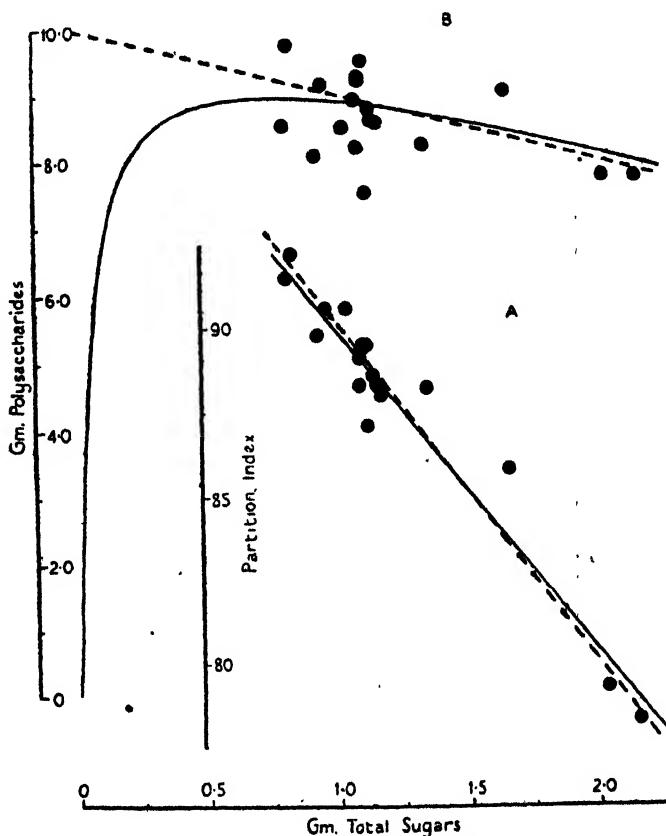


FIG. 2. Relationship between 'partition index' and crystalloid-N content, reproduced from Phillis and Mason (1942). The inserted broken curve was calculated from the broken straight line of Fig. 1.

since an extension of surface must then continue so long as crystalloid-N is available, and this fraction does not disappear. Again, the difficulties involved in treating as one reactant the complex of soluble compounds, of either nitrogen, phosphorus, or sugar, are recognized but not resolved by Phillis and Mason.

Of far greater importance, however, is the employment of the 'partition indices'. The chief attention has been focused by Phillis and Mason on the rectilinear relation existing between 'partition index' and the soluble fraction, and this has been used as a criterion of apolar adsorption; yet it would not seem to be inherent in adsorption phenomena. The regression line in Fig. 2 unquestionably represents with mathematical exactitude the data correlated, yet it should be borne in mind that protein-N content can be predicted from a knowledge of crystalloid-N content with only the same accuracy from this

regression line as from the hyperbola in Fig. 1, a curve which at first sight fits the experimental points with considerably less accuracy. Hence it is self-evident that the improvement in fit when using 'partition indices' is wholly spurious, and due to the method of expressing the results, whereby both the



FIGS. 3 A and B. Fig. 3 A. Relationship between 'partition index' and total sugar content, with regression line, reproduced from Phillis and Mason (1942). The inserted broken line passes through the point: total sugar, 0.0 per cent.; 'partition index', 100. Fig. 3 B. Polysaccharide and total sugar contents, with the curve calculated from the regression line of A and the (inserted) broken line similarly calculated from the broken line in A.

errors and treatment effects are automatically smoothed. This follows from the fact that when 'partition indices' are used, crystalloid-N content appears in both the variables correlated. The extent to which this procedure disguises the true variability of the data is shown by the broken curved line inserted in Fig. 2. This has been calculated from the horizontal broken straight line also inserted in Fig. 1, at the level 4.2 gm. protein-N. The inconsiderable difference between the straight and curved lines drawn in Fig. 2 is now all that remains of the large discrepancy between the curved and straight lines in

Fig. 1, and the great loss in sensitivity entailed in the method of presentation is apparent.

Moreover, points falling on the curved line in Fig. 2 would indicate *complete independence* between protein-N and crystalloid-N contents, and yet the correlation coefficient calculated from the 'partition indices' and crystalloid-N contents would nevertheless be very high indeed. To illustrate this fact, two series of twenty numbers each were taken from a table of random numbers, one series covering the observed range of crystalloid-N in Phillis and Mason's data, and the other the observed range of protein-N. The numbers in these series were paired at random, and 'partition indices' calculated. The experiment was repeated, and the resulting correlation coefficients between 'partition index' and 'crystalloid-N' were  $-0.964$  and  $-0.945$ . The slopes of the regression lines were respectively  $-14.6$  and  $-13.3$ , whilst that of Phillis and Mason's regression is  $-13.5$ . Since in Fig. 2 the actual fitted line and one representing complete independence between protein-N and crystalloid-N contents so nearly coincide, any correlations obtained should be accepted with the greatest caution, or, preferably, the method involving 'partition indices' abandoned altogether.

In view of these facts the correlation coefficient ( $-0.91$ ) extracted by Phillis and Mason from some of the author's data (Richards, 1938) would be unusually low even for random numbers; actually a simple dot diagram relating protein-N and crystalloid-N contents in these data immediately discloses large positional grouping effects due to treatment, and that no regression line, whether hyperbolic or not, can adequately represent the data as a whole. Contrary to their claim then, this experiment can in no way be accepted as evidence in favour of the views of Phillis and Mason.

Neither do their own data supply altogether convincing evidence when the correlation diagram between the contents of the two nitrogen fractions (Fig. 1) is considered. Over the middle part of the observed range of crystalloid-N, which covers most of the observations, protein-N content is quite independent of crystalloid-N and is approximately constant; the points here represent the more normal nutritional treatments of the experiment, yet there is well-defined grouping according to leaf position. At the end of the range representing lower nitrogen contents occur points derived from nitrogen-deficient plants. Here, as might be expected, both fractions fall together; admittedly no discontinuity appears between the lower and higher nitrogen points, at least in so far as the points representing upper leaves of expt. 3 are concerned, but such continuity would naturally be expected on most hypotheses of the mechanism of partition. Finally, there is a group of six points at the high-nitrogen end of the curve, covering a range from 1.5 to 3.0 per cent. crystalloid-N. Over this wide range there is a slight indication of falling protein content, but, regarded as a group, the main characteristic is wide variation in crystalloid-N content associated with nearly constant protein-N. The inserted horizontal line of constant protein-N apparently fits these points almost as well as the falling curve shown, and the straight line and curve of Fig. 2 fit them about

equally well. These points represent data derived from discs punched from leaves of presumably high-nitrogen plants grown at two potassium levels, and sampled either at once or after floating for a shorter or longer time on a high-nitrogen solution. The design of this final experiment would seem to render it extremely likely that the experimental points derived from it should fit coherently on to the high-nitrogen end of the general curve; at least this cannot be regarded as a surprising result in view of the fact that the discs apparently did little else than accumulate excess nitrogen. If hydrolysis of protein did occur at the highest crystalloid-N concentration, this might legitimately be regarded as a toxic effect.

In spite of the wide range of nitrogen content investigated, little disturbance of the normal nitrogen metabolism appears to have been involved in the experiments. While disturbing agencies, such as potassium and phosphorus shortages, were used to some extent, yet deficiency of these elements was never severe. Unless definite interference with normal metabolism be combined with the wide range of nitrogen supply, there is no obvious reason why the various treatments should not provide a reasonably smooth curve, whatever determines the protein-N level. Under these circumstances, moreover, the most likely type of relationship would seem to be a curve resembling that shown by Phillis and Mason's data. As stated above, data (Richards, 1938) based on the use of severe deficiencies of phosphorus and potassium do not provide such apparently simple relationships, and render untenable the view that the 'partition index', and hence protein content, are determined in a simple manner by the concentration of the remaining nitrogenous substances alone.

Fig. 3 reproduces the results for carbohydrates. The original regression line shown in Fig. 3A passes nearly through the point representing no soluble sugar and 'partition index' of 100. A broken line passing through this point and covering the data has been added by the writer; clearly no statistical evaluation of the data could discriminate between the fit of these two lines. Yet any straight line through this particular point transforms back to one of a parallel set of straight lines cutting the axes at  $45^\circ$ , the appropriate one being shown in Fig. 3B, and represents a condition of constant 'labile carbohydrate', i.e. *this broken line represents the partitioning of a constant amount of total carbohydrate into soluble and insoluble fractions in all possible proportions*, the complete antithesis of what should occur in apolar adsorption! The extrapolated portions of the transformed lines in Fig. 3B, with no data to aid in discrimination, are vastly different; clearly these results cannot be legitimately used to support the theory. This difference between the relations of the extrapolated portions of the two lines in Figs. 3A and 3B again emphasizes the insensitivity of the method involving 'partition indices'.

The sets of lines, of which the broken lines in Figs. 3A and 3B are representative members, are, however, of more general interest in the theory of apolar adsorption. If for any reason equilibrium between the insoluble and soluble fractions has not been established, so that the point representing their relation does not lie on the straight regression in Fig. 3A nor on the hyperbola in

Fig. 3B, then in moving towards equilibrium, provided the total amount remains unaltered, in either diagram the *path of the point must be one particular line from the appropriate set*. Moreover, it is easy to show that the asymptote of the falling limb of the hyperbola is itself a particular line from the same set in Fig. 3B: this line corresponds to the one in Fig. 3A running parallel to the actual regression line and passing through the point representing no soluble sugar and 'partition index' of 100. It is immediately apparent therefore that the theory under consideration can apply only when the sum of the contents of the insoluble ( $I$ ) and soluble ( $S$ ) fractions is below a certain limiting magnitude; if this sum is greater than the  $I+S$  value represented by the asymptote itself, the point in Fig. 3B will be situated above the asymptote. Since by internal adjustment between  $I$  and  $S$  the point must move parallel to this line it can never reach an equilibrium value on the hyperbola. As the limiting total content is approached from below, a slight change in the actual value must inevitably lead to a very large change in the ratio of  $I$  to  $S$ , and a large spatial movement on the diagram.

If 'partition indices' are expressed relative to unity instead of 100, the regression line fitted by Phillis and Mason becomes  $\frac{I}{I+S} = a \cdot S + b$ . From this it may be shown that along the hyperbola the relation holds:

$$I+S = \frac{S}{1-b-a \cdot S}.$$

When  $S$  increases indefinitely the limiting value of  $I+S$  becomes that of the asymptote, i.e.  $-1/a$ , the negative reciprocal of the slope of the regression in Fig. 3A. Measurement of these slopes on the diagrams presented by Phillis and Mason leads to the following approximate limiting contents of  $I+S$ : nitrogen, 7.5 per cent.; phosphorus, 0.71 per cent.; and 'labile carbohydrate', 10.9 per cent. These are mathematical limits which can be attained only if negative values of  $I$  are allowed; practically, of course, these are impossible, and a better and rather lower limit is given by the value of  $S$  on the hyperbola when  $I = 0$ . Substituting in either of the above equations the limit becomes  $-b/a$ . The revised limits, as nearly as they can be estimated from the diagrams, are as follows: nitrogen, 7.1 per cent.; phosphorus, 0.66 per cent.; and carbohydrate, 10.8 per cent.

It is noteworthy that in all three classes of substances some of the higher observed  $I+S$  values approach these limits very closely indeed, if they do not actually exceed them. Hence in all cases a slight further increase of the observed total content should result in complete disappearance of the insoluble fraction—unless indeed the form of the curve relating  $I$  to  $S$  changes radically in the extrapolated region; this, however, in itself would be strong evidence against the theory as advanced by Phillis and Mason. It is indeed a remarkable example of physiological co-ordination if, of a considerable variety of substances, the maximum amount normally accumulated is so nicely adjusted to the individual requirements of these adsorption curves.

It is evident, on the other hand, that all points derived from the carbohydrate and phosphorus data are placed by the method of analysis along the falling limb of the hyperbola, and it has been shown that here, except quite close to the vertex, a nearly constant total amount is partitioned in very variable ratios of  $I$  to  $S$ . The chief characteristics of both these sets of data then are (1) inconsiderable variation in the sum  $I+S$ , and (2) wide variation in the ratio of  $I$  to  $S$ . Clearly in a large measure  $I$  depends mathematically on  $S$  simply because  $I+S$  has a greater tendency to constancy than either of its constituent fractions, which are therefore negatively correlated; the hyperbolic fit succeeds because along one of the limbs  $I+S$  again approximates to constancy, and not because this particular type of curve is characteristic of apolar adsorption. In order to avoid this simple arithmetical dependence between  $I$  and  $S$  it is necessary to examine data at lower total contents of phosphorus and carbohydrate, so that the rising limb of the hypothetical hyperbola is also explored. Even were this done the same difficulties would presumably arise as are found in the nitrogen data of Phillis and Mason, since as  $I+S$  falls towards zero both fractions must inevitably eventually fall, simulating the other half of the adsorption curve.

*Water content.* Mason and Phillis (1942) claim that previous studies, and particularly those of Gregory and Richards (1929) and of Richards and Shih (1940 *a* and *b*), on the relation between potassium supply and water content, have been vitiated by failure to appreciate the importance of a 'size factor', and that where increased supply does not lead to marked increases in growth the effect of potassium is to increase water content, but where large increases in size occur it is likely to decrease succulence. The controlling factor is 'environmental aridity'. Where soil or air humidity is high an increase in growth due to potassium is accompanied by increased succulence, but where humidity is low increased water strain resulting from increased size may outweigh the potassium effect and lower the water content. Sand cultures are particularly prone to high aridity round the roots.

It is quite possible that under their conditions such a factor might be of importance. Their work was performed in the tropics, and experiments are described in which cotton was grown to a large size, in some instances well over 300 gm. dry weight, in pots holding only 10 lb. of sand. In the experiments at this Institute, on the contrary, shortage in water supply was carefully avoided. The experiments were done in a temperate climate, pots holding 30 lb. of sand were used, and in the experiment of Richards and Shih the dry weight of the crop per pot in the largest treatment on the last sampling occasion was 56 gm., and on the first occasion only 4.4 gm. The crucial point is that these small plants showed *either considerably increased or considerably decreased* leaf succulence at one-ninth the normal potassium level, according as the calcium : sodium ratio was low or high. This general difference held over a fairly wide range of phosphorus nutrition. Moreover, the reduction in dry weight due to potassium starvation was similar in both high-sodium and high-calcium series, being at the high-phosphorus level about 18 per cent. at

the first sample, 30 per cent. at the second, and 52 per cent. at the third. Under these circumstances the 'size factor' cannot have assumed the importance in this work that Mason and Phillis would ascribe to it.

Mason and Phillis's data are interpreted entirely along the lines of an assumed positive potassium effect combined with this 'size effect', and possible effects of other elements and of carbohydrate level are disregarded. Nevertheless it is evident that many of the results they ascribe to the 'size factor' might quite well be due to such neglected causes; to pursue this argument in detail would occupy too much space.

Finally, it may be asked, if in pot culture a 'size factor' is of so great importance in determining the direction of deviation in water content with potassium deficiency, why is the same factor apparently inoperative with variation in the supply of other nutrients, as nitrogen and sulphur?

In a later series of papers the same authors attempted to assess the importance of the salts present in the leaf in determining water content, and arrived at conclusions which they state are not in harmony with those of Richards and Shih (1940 *a* and *b*). Mason and Phillis (1942 *a*) first investigated over several days the changes in water content of discs punched from cotton leaves and floated either on water, a nutrient solution, or calcium chloride solution. Besides water content, observations were made of the changes in specific conductivity of the expressed sap, and in its freezing-point depression. An 'estimate of salt content' was also made, namely the product of conductivity and water content (gm. water per 100 gm. dry weight). Since the changes in water content followed those in 'salt content' much more closely than those in conductivity or in freezing-point depression, it was concluded that water uptake is conditioned by the amount of salts rather than their concentration in the sap. The suggestion was made that salt increases the hydration capacity of leaf proteins. The questionable nature of the method of dealing with the data on which their conclusions rested was to some extent at least appreciated by the authors, and indeed in a later contribution (Phillis and Mason, 1943) a frank withdrawal was made, and it was concluded that the evidence did not enable a decision to be taken as to whether conductivity of the sap or amount of salt is the more important.

In spite of this, in a still later paper (Mason and Phillis, 1943), the same method of dealing with the data was again employed, hence it may be advisable to indicate some of the more obvious shortcomings of the method. In the first place, by substituting 'salt content' (i.e. conductivity  $\times$  water content) for conductivity a substantial correlation with water content must necessarily appear. As an illustration, twenty pairs of numbers were taken from a table of random numbers, one set covering the observed range of conductivity in the experiment under discussion, and the other the observed range of water content, both expressed on the basis of protein-N. These two sets were quite insignificantly related, what relationship there was being inverse ( $r = -0.18$ ). The products of these pairs were then correlated with the set representing water, the coefficient reaching the height of  $+0.78$ . Since coefficients of this



order are obtained when the primary variates are deliberately taken at random, the correlations between 'salt' and water obtained by Mason and Phillis are of no remarkable magnitude (incidentally the extremely high value given by them for the coefficient, when both variates are expressed in terms of protein-N, is clearly erroneous).

Again, correlating water content with 'salt content' is equivalent to fitting the straight line regression:  $W = a.S + b$ . Since  $S = C.W$ , this line transforms back to the rectangular hyperbola  $W = \frac{b}{1-a.C}$ , relating simply the two measured variables. This curve has two branches, the delimiting value of  $C$  being  $1/a$ . Since  $W$  is negative when  $C$  exceeds this value (both constants being positive in their experiment), only the other branch of the curve is in question, and this is concave to the water-content axis. Thus in fitting water content by straight lines to both conductivity and 'salt', relative to conductivity alone there is open the choice either of a straight line fit or of a curve concave to the water-content axis.

The 'salt' transformation then may improve the simple correlation coefficient in two ways: (1) by straightening the regression line and (2) in a purely algebraic manner by introducing a factor common to both variates. The magnitude of the correlation coefficient obtained is the sole criterion used by Mason and Phillis in discriminating between the effects of conductivity and 'salt', although there would appear to be no grounds for assuming that the relationship with either characteristic is rectilinear. Even if the criterion of rectilinearity is accepted it would seem that the improvement due to the use of the 'salt' transformation is spurious. The primary correlation between water and conductivity is not very good, the coefficient being  $+0.48$  when the variables are expressed in terms of dry weight; there seems, however, to be no evidence of curvature in the relationship, as may be seen from the correlation diagram (Mason and Phillis, 1943, Fig. 4). The corresponding coefficient between water and 'salt' contents is  $+0.87$ , not a high value when the extent of the correlation gratuitously introduced is considered; moreover the correlation diagram (Mason and Phillis, 1943, Fig. 2) now shows distinct evidence of curvature, this curve being convex to the water-content axis. Hence even if there should be slight concave curvature in the relationship with conductivity, the 'salt' transformation has distinctly introduced a greater curvature in the opposite direction. The same remarks apply when the variables are expressed in terms of protein-N. Hence there are no rational grounds for using the 'salt' transformation in this experiment; its use confuses the main issue, which is to determine the extent to which the water-content data are accounted for by other *measured* variables, in this case conductivity alone. If the correlation is low and is not improved by fitting a curved regression by standard statistical methods, then either experimental errors are large or else the two variables are not simply related. Multiplying the variables alters the shape of the regression line but cannot reduce the real errors of the experiment, hence in these circumstances the resulting improvement in fit will be wholly an artifact.

The impossibility of drawing valid conclusions by such methods as to the relative importance of conductivity and 'salt content' in determining water content may be readily appreciated from a consideration of the normal statistical practice adopted when a basis for such judgements is required. In such an event partial correlation coefficients of the supposed dependent variate with each independent variate are determined after eliminating effects ascribable to correlation with the other independent variate. If now 'salt' be calculated by multiplying conductivity and water, and we choose to express all values logarithmically, clearly both partials must be perfect, that with 'salt' being positive and that with conductivity negative. When it is desired to investigate the effect of salt content on water content, therefore, independent methods of estimating both should be sought.

The experiment under consideration was mainly factorial in design, the variables being phosphorus supply, potassium supply, and the calcium : sodium ratio; it therefore followed closely the design used by Richards and Shih (1940). The conclusion was reached that the results did not indicate any specific effects of the individual elements on water content, and in this respect were at variance with those of Richards and Shih. To account for this supposed difference it was pointed out that in Mason and Phillis's experiment only elements in solution in the sap were considered, not the total amount, and moreover that the earlier experimenters neglected to consider the 'size effect'. This latter criticism has already been dealt with. It is true that Richards and Shih found apparent differential effects of the elements, but they showed also that the apparent effect of any one element changed with the age of the plant, and was not even nearly constant between the nutritional sub-groups of their experiment; thus sodium and phosphorus might have no apparent effect on succulence at high-potassium levels, but large effects at low-potassium levels. An elaborate analysis of their data led them to the conclusion that no reliance as a constant could be placed in the regression coefficient obtained at any one time or under one set of conditions, and that one considerable disturbing factor was carbohydrate level. In these circumstances the question as to whether under really comparable conditions differential effects of the elements exist was perforce left undecided.

Mason and Phillis's conclusion was reached from visual inspection of the correlation diagram between water and 'salt' contents, a diagram which, as has been shown, appeals to the eye solely on account of a spurious correlation introduced by the application of an unjustifiable mathematical device. When it is borne in mind that in actual fact, on a dry-weight basis, less than a quarter of the total variance in the water-content data of the experiment could be accounted for by conductivity, the only other variable measured, and on a protein-N basis less than a third, it calls for no surprise that considerable evidence of possible differential effects was not forthcoming. Meanwhile, in view of the poor fit obtained by use of conductivity measurements alone, which presupposes the absence of differential effects of the various ions, it is at least possible that real improvement might have resulted from observation

of the actual concentrations of the single elements and using these values for fitting individual constants. It may be remarked that by so doing Richards and Shih were able to account for between 80 and 96 per cent. of the total water variance.

The author, however, cannot accept the statement that Mason and Phillis's data show 'no evidence of a specific effect of any single element on hydration'. Thus the slope of the line connecting the mean values of 'salt' and water per gm. protein-N, representing the regression of water on 'salt', is about 75 per cent. greater among the phosphorus-level sub-groups than among the calcium:sodium ratio sub-groups; in terms of dry weight a similar difference holds although all the lines are curved. This is in very good agreement with the results of Richards and Shih. Mason and Phillis obtained an effect on water content presumably due largely to the potassium ion, whereas Richards and Shih found no such direct evidence in their data. The latter investigators attributed the absence of such effect as probably due to the correlation existing in their data between potassium and carbohydrate level; they further suggested that in order to observe direct potassium effects it would be necessary experimentally to destroy this correlation, as by working under reduced light. It is therefore interesting to note that Mason and Phillis, in order to minimize the 'size effect' which they consider to be important, approximated to this condition by subjecting their plants when three weeks old to a day-length of only six hours, this photoperiod being maintained for a further four weeks, until the time of harvest. At the same time it may be noted that of the three potassium levels they used, the highest (500 p.p.m.) must be regarded as excessive supply. The other two alone explore the region of deficiency covered by Richards and Shih; moreover only the data expressed on a dry-weight basis are comparable with the earlier work. Inspection of Mason and Phillis's correlation diagram between 'salt' and water contents shows for the means of these two potassium levels a considerable difference in 'salt content' unaccompanied by appreciable difference in water content. Within the range of nutrient supply in which the two sets of data are comparable, therefore, the results agree in assessing phosphorus as the element showing most apparent effect, potassium least, and sodium and calcium intermediate.

### CONCLUSION

The above examination of the methods used by Mason and Phillis in analysing their data demonstrates that many of the conclusions reached and theories advanced are not supported by those data. In so far as previous attempts at analysis were held by them to be inadequate or erroneous, there is not the least evidence presented to warrant such a conclusion. On the other hand, the theories themselves rest on inadequate experimental foundations, and it has been pointed out that the simple relations deduced are spurious, resulting from unjustifiable handling of the primary data. Without further experimental evidence, supplemented by adequate statistical analysis, it would

appear that both the water-content relations and the partitioning between soluble and insoluble fractions of nitrogen, phosphorus, and carbohydrate are more complex phenomena than envisaged by Mason and Phillis, as indeed the work carried out in this Institute has amply demonstrated.

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#### ADDENDUM

While the above paper was passing through the press two further contributions appeared from Mason and Phillis on the subjects under discussion.<sup>1</sup> These necessitate little modification of the present review. It may be noted, however, that further work on the partitioning of 'labile carbohydrate' has led to the abandonment of the view that apolar adsorption is here the primary determinant, though the theory is still held to account for the partitioning of nitrogen and probably of phosphorus. The problem of the 'size factor', in relation to water content and potassium supply, has also been further investigated. Pronounced effects of age of plant were found on water content, even under conditions of good moisture supply, together with other effects incompatible with the 'size factor' operating *via* simple water strain. Such phenomena again point to the possibility that carbohydrate status, with effects on wall extensibility, as envisaged by Richards and Shih (1940), may well be a dominant constituent of the 'size effect', and one at least meriting consideration.

<sup>1</sup> Phillis, E., and Mason, T. G., 1943: Studies on Foliar Hydration in the Cotton Plant. V. A further Experiment with Potassium. *Ann. Bot., N.S.*, vii. 391. Mason, T. G., and Phillis, E., 1943: Studies on the Partition of the Mineral Elements in the Cotton Plant. IV. More about Nitrogen, Phosphorus, and Labile Carbohydrate. *ibid.* 399.



# Wilt of Cacao Fruits (*Theobroma Cacao*)

## III. Changes in Mineral Content during Development

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With five Figures in the Text

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### I. INTRODUCTION

IN the course of an investigation into the causes of wilt of cacao fruits, field observations indicated that the fruits are susceptible to wilt from purely physiological causes only during the early part of their development (Humphries, 1943). By following successive changes in dry weight, water, carbohydrate, and fat content during the ontogeny of the fruit, a relationship was shown to exist between the internal condition and the wilt susceptibility. It was shown that the growth of the fruit may be divided into two distinct phases, the first occupying about 75 days, during which period the fruit is liable to physiological wilt at any age (Humphries, 1943 *a*). Several lines of evidence indicated that wilt was due to competition for water and nutrients between developing fruits. It was considered, therefore, that an ontogenetical survey of the changes in content of mineral elements of the fruit would be of value. The results of such a survey are discussed in the present paper.

### II. METHODS AND PROCEDURE

When fruits were gathered for the carbohydrate investigation, representative sub-samples were taken by slicing the fruits of each age group longitudinally

and these were dried at 105° C. and put aside for subsequent ash analysis. Each sample was subdivided into wall and pulp as in the case of the carbohydrate samples. Insufficient material was available for analysis of the pulp of fruits younger than 52 days, but figures were obtained for the wall from 25 days onwards. The following methods of analysis were employed: *phosphorus* was determined by the molybdate blue method using  $\alpha$ -amino-naphtho-sulphonic acid as reducing agent, *potassium* by precipitation with sodium

TABLE I

*Mineral Constituents in the Pulp at Successive Stages of Development expressed as a Percentage of the Residual Dry Weight*

| Days. | Carbohydrate<br>and fat<br>(% D.W.) | Total ash. | P    | K    | Ca   | Mg   |
|-------|-------------------------------------|------------|------|------|------|------|
| 52    | 15.75                               | 8.36       | 0.52 | 2.88 | 1.15 | 1.33 |
| 57    | 19.72                               | 8.74       | 0.53 | 3.11 | 0.75 | 1.14 |
| 63    | 17.70                               | 9.30       | 0.58 | 3.17 | 0.62 | 0.75 |
| 68    | 16.84                               | 9.25       | 0.52 | 3.24 | 0.69 | 0.83 |
| 78    | 15.32                               | 10.07      | 0.57 | 3.08 | 1.02 | 0.78 |
| 84    | 17.74                               | 9.70       | 0.56 | 3.18 | 0.90 | 0.57 |
| 87    | 27.07                               | 11.07      | 0.61 | 3.65 | 0.97 | 0.69 |
| 93    | 32.20                               | 10.04      | 0.59 | 3.45 | 0.77 | 0.65 |
| 99    | 45.16                               | 11.38      | 0.70 | 3.83 | 0.67 | 0.81 |
| 107   | 47.06                               | 12.09      | 0.73 | 4.04 | 0.61 | 0.83 |
| 122   | 54.75                               | 13.81      | 0.89 | 4.70 | 0.59 | 0.92 |
| 143   | 62.68                               | 16.02      | 1.05 | 5.50 | 0.67 | 1.13 |
| 170   | 66.73                               | 12.41      | 1.20 | 3.99 | 0.49 | 1.19 |

cobaltinitrite and subsequent oxidation by standard permanganate, *calcium* by precipitation as oxalate and oxidation by standard permanganate, and *magnesium* by weighing as the pyrophosphate.

### III. CHANGES IN MINERAL CONSTITUENTS OF THE PULP EXPRESSED ON A RESIDUAL DRY WEIGHT BASIS

There is a gradual accumulation of carbohydrate material in the wall and a very considerable accumulation of carbohydrate and fat in the pulp after a certain stage (Humphries, 1943 *a*), hence the results are here expressed on a *residual* dry weight basis (cf. Mason and Maskell, 1928). The amounts of total ash and the separate ash constituents of the pulp, expressed as a percentage of the residual dry weight (i.e. total dry weight minus glucose, fructose, sucrose, starch, and fat), are shown in Table I and are presented graphically in Fig. 1, where the values expressed on a dry weight basis are also shown for comparison. (No figures were available for the mucilage content of the wall during development.) There is a continuous increase of total ash expressed as a percentage of residual dry weight in the pulp up to the time that the fruit is fully grown and is commencing to ripen (143 days), after which a considerable decrease occurs. A similar relationship was found for potassium, where there is a continuous increase in percentage amount up to 143 days and a decrease at

maturity. In the case of both total ash and potassium, expressed on a total dry weight basis, there is a continual decrease from about 84 days onwards. The amount of phosphorus as a percentage of residual dry weight increases slowly up to 93 days and then continues at an accelerated rate until maturity

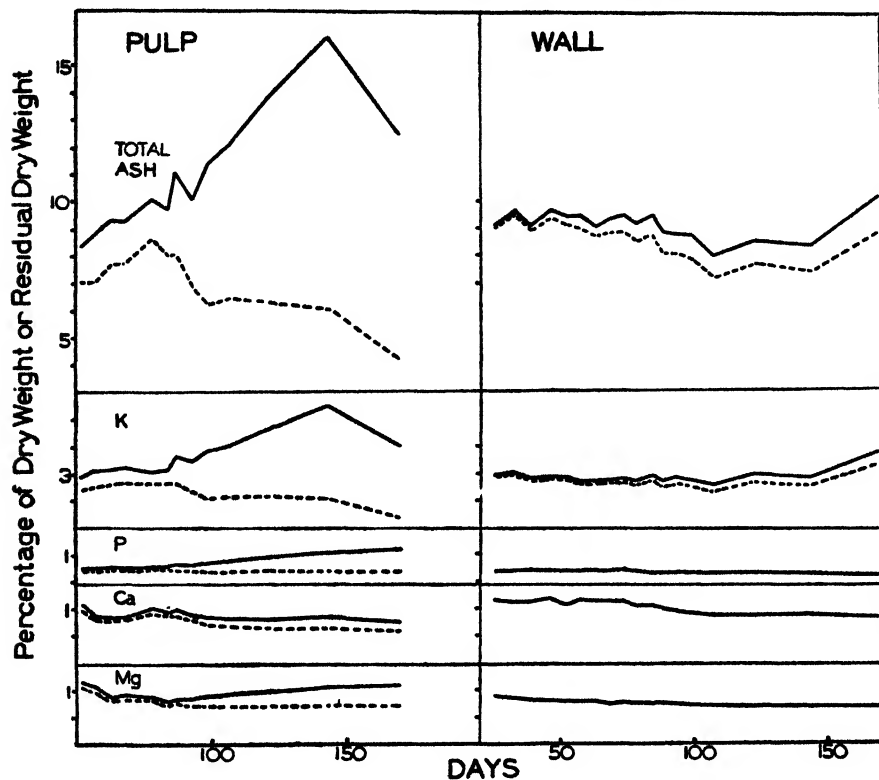


FIG. 1. Changes in percentage content of total ash, potassium, phosphorus, calcium, and magnesium in the wall and pulp during development. The results expressed on a residual dry weight basis are indicated by continuous lines, and on a dry weight basis by broken lines.

with no decline during the ripening period as in the case of potassium. Both calcium and magnesium show a slight decline in the initial stages whether expressed as a percentage of total dry weight or residual dry weight. Calcium reaches a maximal value between 78 and 87 days and then declines slowly. Magnesium increases slowly but continuously after 93 days on a residual dry weight basis, but remains practically constant after this date on a total dry weight basis.

#### IV. CHANGES IN MINERAL CONSTITUENTS OF THE WALL EXPRESSED ON A RESIDUAL DRY WEIGHT BASIS

The carbohydrate changes in the wall of the fruit are of much smaller magnitude than in the pulp, consequently there is not such a striking difference



in the case of the wall data between the two modes of expression. The data are presented in Table II and are also plotted in Fig. 1 on the same scale as the pulp data. The total ash as a percentage of total dry weight or residual dry weight shows a downward trend until ripening commences, when there is

TABLE II

*Mineral Constituents in the Wall at Successive Stages of Development expressed as a Percentage of the Residual Dry Weight*

| Days. | Carbohydrate<br>(% D.W.). | Ash.  | P    | K    | Ca   | Mg   |
|-------|---------------------------|-------|------|------|------|------|
| 25    | 1.09                      | 9.12  | 0.36 | 2.91 | 1.30 | 0.74 |
| 32    | 1.17                      | 9.62  | 0.35 | 3.00 | 1.22 | 0.67 |
| 39    | 2.05                      | 9.10  | 0.35 | 2.75 | 1.22 | 0.60 |
| 46    | 3.12                      | 9.66  | 0.34 | 2.84 | 1.34 | 0.59 |
| 52    | 3.87                      | 9.48  | 0.33 | 2.83 | 1.17 | 0.53 |
| 57    | 4.89                      | 9.46  | 0.32 | 2.69 | 1.30 | 0.54 |
| 63    | 4.16                      | 9.10  | 0.38 | 2.67 | 1.27 | 0.53 |
| 68    | 5.26                      | 9.34  | 0.33 | 2.73 | 1.25 | 0.48 |
| 73    | 6.36                      | 9.47  | 0.34 | 2.79 | 1.21 | 0.51 |
| 78    | 7.04                      | 9.11  | 0.30 | 2.70 | 1.06 | 0.46 |
| 84    | 7.65                      | 9.47  | 0.28 | 2.90 | 1.06 | 0.45 |
| 87    | 8.91                      | 8.82  | 0.28 | 2.68 | 0.95 | 0.45 |
| 93    | 8.80                      | 8.79  | 0.26 | 2.81 | 0.88 | 0.42 |
| 99    | 10.79                     | 8.73  | 0.23 | 2.69 | 0.78 | 0.39 |
| 107   | 9.54                      | 7.93  | 2.21 | 2.52 | 0.69 | 0.37 |
| 122   | 10.39                     | 8.50  | 0.21 | 2.90 | 0.69 | 0.37 |
| 143   | 11.27                     | 8.32  | 0.21 | 2.79 | 0.71 | 0.34 |
| 170   | 12.43                     | 10.23 | 0.18 | 3.86 | 0.62 | 0.36 |

a rapid increase to maturity. Potassium shows a very slight downward trend throughout except during the ripening period, when there is a big increase. Phosphorus, calcium, and magnesium exhibit a downward trend throughout the growth of the fruit, but during the last 50 days the decrease is very slight in each case.

#### V. ABSOLUTE CONTENT OF MINERAL CONSTITUENTS OF THE WALL AND THE PULP AT SUCCESSIVE STAGES OF DEVELOPMENT

The amount of any particular mineral element at any stage in development may be seen from Tables III and IV, where the absolute amounts of total ash, phosphorus, potassium, calcium, and magnesium in the wall and in the pulp at different ages are shown. In spite of the greater dry weight of wall over pulp at all stages of development the total phosphorus content of the pulp is much greater than that of the wall when the fruit is mature, there being a very large increase of phosphorus in the pulp during the preripening and ripening stages. The data indicate that there is an actual loss of phosphorus from the wall during the ripening period and it is possible that this is transferred to the pulp. The total phosphorus content of the wall exceeds that of the pulp up till about 107 days, but after this the position is reversed. The total potassium and total calcium contents of the wall exceed that of the pulp

throughout development. The total magnesium content of the wall is greater than that of the pulp during all stages except at maturity when it slightly exceeds the wall value.

An indication of the relative uptake of minerals by the wall and pulp may

TABLE III

*Mineral Constituents (mg.) in the Wall of a single Fruit at Successive Stages of Development*

| Days. | Ash.    | N     | P    | K       | Ca    | Mg    |
|-------|---------|-------|------|---------|-------|-------|
| 25    | 17.0    | 5.2   | 0.7  | 5.4     | 2.4   | 1.4   |
| 32    | 41.3    | 11.8  | 1.5  | 12.9    | 5.2   | 3.1   |
| 39    | 62.2    | 16.9  | 2.4  | 18.8    | 8.4   | 4.1   |
| 46    | 117.9   | 29.7  | 4.2  | 34.6    | 16.3  | 7.1   |
| 52    | 185.8   | 48.1  | 6.5  | 55.5    | 22.9  | 10.4  |
| 57    | 312.3   | 81.6  | 10.6 | 88.7    | 42.9  | 18.0  |
| 63    | 380.2   | 102.0 | 15.8 | 111.8   | 53.3  | 22.1  |
| 68    | 503.9   | 126.0 | 17.7 | 147.6   | 67.6  | 25.8  |
| 73    | 598.7   | 140.4 | 21.2 | 176.5   | 76.3  | 32.6  |
| 78    | 789.4   | 180.8 | 26.1 | 233.7   | 92.0  | 40.5  |
| 84    | 1,043.0 | 231.3 | 31.3 | 318.6   | 116.8 | 48.9  |
| 87    | 1,259.9 | 287.1 | 39.1 | 382.9   | 135.8 | 64.3  |
| 93    | 1,546.3 | 352.8 | 46.3 | 494.5   | 154.4 | 74.4  |
| 99    | 1,887.5 | 399.8 | 49.8 | 581.3   | 168.0 | 83.3  |
| 107   | 2,473.7 | 538.2 | 66.3 | 784.6   | 214.6 | 114.5 |
| 122   | 3,269.0 | 665.0 | 80.6 | 1,114.5 | 263.8 | 144.8 |
| 143   | 3,630.7 | 702.6 | 90.2 | 1,215.2 | 309.1 | 151.3 |
| 170   | 4,539.1 | 805.5 | 79.7 | 1,711.5 | 275.3 | 158.8 |

also be obtained by considering the incremental increases of particular elements over short periods of time. In Fig. 2 are shown the increments of potassium and phosphorus in wall and pulp at 10-day intervals. In both parts the greatest increase of these elements occurs between 100 and 110 days. During this period the uptake of phosphorus in the pulp greatly exceeds that of the wall, and the potassium uptake of the pulp is practically the same as that of the wall. The increments of phosphorus in the pulp increase again from 140 days onwards as also do the increments of potassium in the wall.

#### VI. RELATIVE RATE OF UPTAKE OF MINERAL CONSTITUENTS BY THE WALL AND PULP AT SUCCESSIVE STAGES OF DEVELOPMENT

By plotting the absolute amounts of the various mineral constituents on a logarithmic scale against time it is possible to estimate their relative rates of uptake. These are shown in Fig. 3. The relative rate of uptake of potassium by the wall is constant up to between 50 and 60 days, when a lower but constant rate of uptake is established until about 107 days. The uptake of potassium by the pulp, on the other hand, shows a constant rate from 50 to 107 days, and this rate is the same as that of the first phase in the wall. The same relationship holds for the other elements studied, as Fig. 3 shows where the regression lines have been fitted to the data. The regression coefficients with their standard errors are set out in Table V. In all cases the agreement

between calculated and observed values is high, as shown by the correlation coefficients. By inspection it is clear that for each of the elements in turn the relative rate of uptake in the wall between 25 and 57 days is not significantly different from the relative rate of uptake in the pulp between 52 and 107 days.

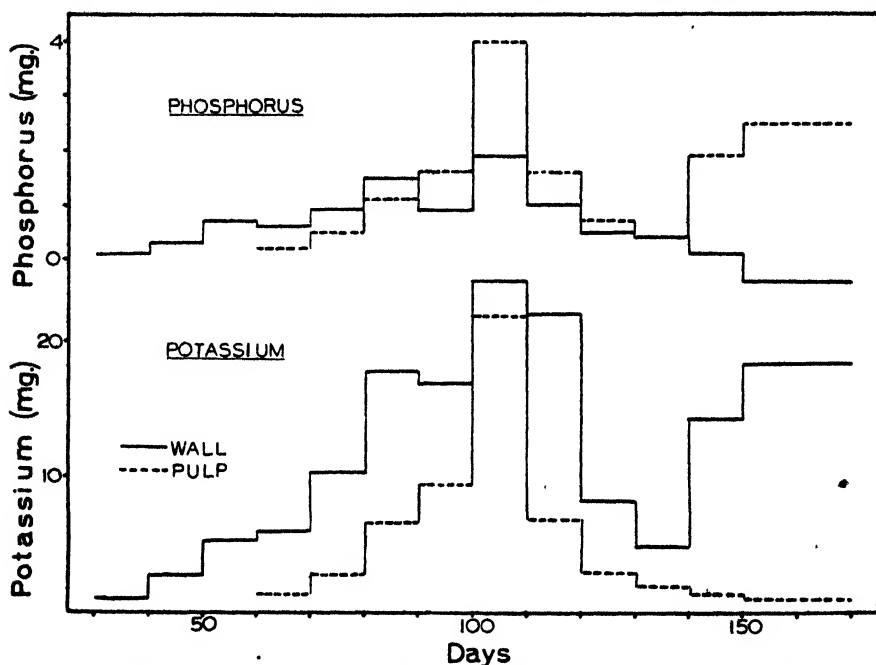


FIG. 2. Increments (mg.) of phosphorus and potassium in the wall and pulp at successive 10-day intervals.

TABLE IV

*Mineral Constituents (mg.) in the Pulp of a single Fruit at Successive Stages of Development*

| Days. | Ash.    | N     | P     | K     | Ca   | Mg    |
|-------|---------|-------|-------|-------|------|-------|
| 52    | 11.1    |       | 0.7   | 3.8   | 1.5  | 1.8   |
| 57    | 17.9    |       | 1.1   | 6.4   | 1.5  | 2.4   |
| 63    | 28.2    |       | 1.8   | 9.6   | 1.9  | 2.3   |
| 68    | 43.8    |       | 2.5   | 15.3  | 3.3  | 3.9   |
| 78    | 145.0   |       | 8.2   | 44.3  | 14.6 | 11.2  |
| 84    | 178.0   | 52.2  | 10.3  | 58.4  | 16.6 | 10.4  |
| 87    | 293.0   | 87.7  | 16.2  | 96.9  | 25.7 | 19.3  |
| 93    | 390.9   | 132.6 | 23.1  | 134.4 | 30.0 | 25.3  |
| 99    | 536.6   | 196.1 | 32.7  | 180.6 | 31.4 | 38.4  |
| 107   | 1,212.2 | 433.7 | 72.9  | 405.6 | 60.9 | 83.4  |
| 122   | 1,494.4 | 571.5 | 96.1  | 508.0 | 63.3 | 99.5  |
| 143   | 1,597.3 | 603.7 | 105.1 | 547.6 | 66.9 | 112.8 |
| 170   | 1,786.2 | 951.5 | 172.0 | 574.4 | 71.1 | 172.2 |

It is also shown that the relative rates of uptake of nitrogen, phosphorus, potassium, and calcium are not significantly different from one another. The

relative rates of uptake of magnesium, on the other hand, while being similar in both wall (first phase) and pulp, are significantly lower than in the case of the other elements. The rate of uptake of any elements by the wall in the second phase (57 to 107 days) is in all cases significantly lower than the rate in the

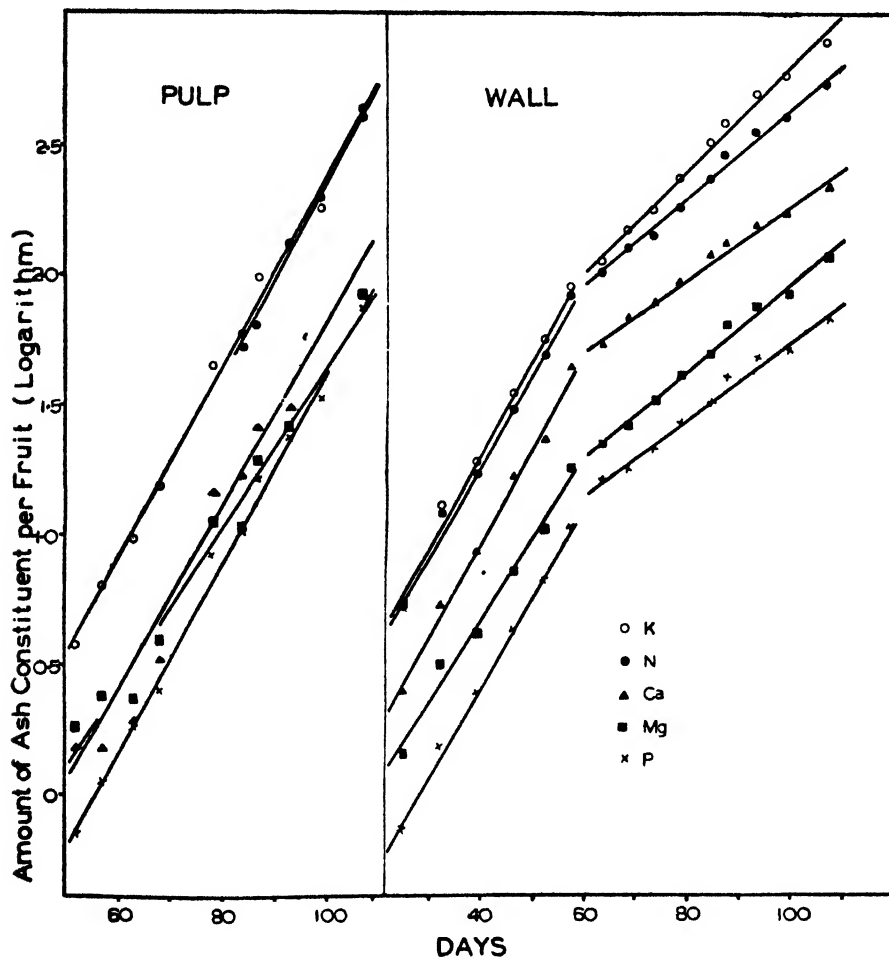


FIG. 3. The absolute amount of nitrogen, phosphorus, potassium, and calcium in the wall and pulp at successive stages of development, plotted logarithmically. For explanation see text.

first phase of the wall. In this second phase the rates of uptake of calcium and phosphorus are closely similar, but the relative rates of uptake of potassium and magnesium are both significantly greater than in the case of calcium and phosphorus; moreover the rates of uptake of potassium and magnesium are significantly different from one another, potassium having the greater rate. The regression coefficient for rate of potassium uptake (0.01969) in the second phase is not very different from the rate of dry matter increase (0.02070) reported previously (Humphries, 1943 a). Thus while the rate of uptake of all

the elements studied, except magnesium, is the same as the rate of uptake of dry matter up to the time of commencement of embryo division (50 to 60 days), only the relative rate of uptake of potassium is maintained at this level subsequently, the rate for the other elements being significantly lower.

#### VII. THE MINERAL CONSTITUENTS OF THE RIPE BEAN

The actual ash analyses for four different ages of wall and pulp are presented in Table VI. The mineral constituents here considered, namely

TABLE V

*Regression Coefficients and their Standard Errors for the Relative Uptake of Mineral Elements by the Wall and the Pulp*

|    | Days.  | Regression coefficient | Standard error. | Correlation coefficient. |
|----|--------|------------------------|-----------------|--------------------------|
| N  | 25-57  | 0.03541                | ±0.0026         | 0.984                    |
| P  | 25-57  | 0.03548                | ±0.0007         | 0.998                    |
| K  | 25-57  | 0.03631                | ±0.0013         | 0.996                    |
| Ca | 25-57  | 0.03732                | ±0.0014         | 0.996                    |
| Mg | 25-57  | 0.03180                | ±0.0015         | 0.993                    |
| N  | 84-107 | 0.03734                | ±0.0055         | 0.972*                   |
| P  | 52-107 | 0.03706                | ±0.0004         | 0.998                    |
| K  | 52-107 | 0.03663                | ±0.0009         | 0.997                    |
| Ca | 52-93  | 0.03725                | ±0.0028         | 0.978                    |
| Mg | 52-107 | 0.03068                | ±0.0015         | 0.988                    |
| N  | 57-107 | 0.01685                | ±0.0004         | 0.997                    |
| P  | 57-107 | 0.01466                | ±0.0005         | 0.994                    |
| K  | 57-107 | 0.01969                | ±0.0005         | 0.997                    |
| Ca | 57-107 | 0.01413                | ±0.0005         | 0.993                    |
| Mg | 57-107 | 0.01653                | ±0.0010         | 0.983                    |

TABLE VI

*Ash Constituents (% of Total Ash) in the Wall and Pulp at Different Stages of Development*

| Days.   | Wall.                         |                  |      |     |        | Pulp.                         |                  |      |      |        |
|---------|-------------------------------|------------------|------|-----|--------|-------------------------------|------------------|------|------|--------|
|         | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO  | MgO | Total. | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO  | MgO  | Total. |
| 57 . .  | 7.8                           | 34.2             | 19.2 | 9.6 | 70.8   | 13.8                          | 42.9             | 12.0 | 21.7 | 90.4   |
| 87 . .  | 7.1                           | 36.6             | 15.0 | 8.5 | 67.2   | 12.6                          | 39.8             | 12.2 | 10.4 | 75.0   |
| 122 . . | 5.6                           | 41.0             | 11.3 | 7.3 | 65.2   | 14.7                          | 41.0             | 5.9  | 11.0 | 72.6   |
| 170 . . | 4.0                           | 45.4             | 8.5  | 5.8 | 63.7   | 22.0                          | 38.7             | 5.6  | 15.9 | 82.2   |

potassium, calcium, magnesium, and phosphorus in the form of their oxides, constitute between 60 and 70 per cent. of the total ash of the wall, whereas in the pulp these account for 70 to 90 per cent. of the total ash. The pulp consists of a white tissue surrounding the testa, the testa itself, and the kernel (embryo and cotyledons) of the bean. Previously published ash analyses of cacao beans indicate that the kernel is even richer in these particular elements than the rest of the pulp. Some analyses are shown in Table VII. It will be

noticed that in all cases, except that of Zeller (1911), the percentage of phosphorus is particularly high and that in two cases the four elements account for over 90 per cent. of the ash. Similar evidence was obtained in the case of the variety of cacao used in the present work. Some samples of nearly ripe fruits were collected and white tissue and testa surrounding the kernel were

TABLE VII

*Analyses of the Ash of the Cacao Bean*

| Author.                             | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO  | MgO   | Total. |
|-------------------------------------|-------------------------------|------------------|------|-------|--------|
| Harrison (1896) (Calabacillo) . . . | 31.82                         | 35.77            | 2.29 | 13.76 | 83.64  |
| Harrison (1896) (Forastero) . . .   | 42.92                         | 26.80            | 4.31 | 18.64 | 92.67  |
| Zeller (1911) . . . . .             | 23.77                         | 29.38            | 6.83 | 12.31 | 72.29  |
| Couturier . . . . .                 | 30.80                         | 36.56            | 6.80 | 16.77 | 90.93  |

TABLE VIII

*Ash Constituents (% of Total Ash) of Ripe and Nearly Ripe Cacao Beans*

| Sample. | Kernel of Bean.               |                  |     |      |        | Pulp (mainly testa).          |                  |      |      |        |
|---------|-------------------------------|------------------|-----|------|--------|-------------------------------|------------------|------|------|--------|
|         | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO | MgO  | Total. | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | CaO  | MgO  | Total. |
| A . . . | 29.5                          | 43.4             | 7.1 | 17.9 | 97.9   | 9.5                           | 41.2             | 22.2 | 16.6 | 89.5   |
| B . . . | 25.3                          | 41.2             | 8.0 | 16.9 | 91.4   | 4.4                           | 53.1             | 10.0 | 8.4  | 75.9   |
| C . . . | 27.9                          | 40.4             | 7.4 | 19.0 | 94.7   | 6.0                           | 53.1             | 14.0 | 9.6  | 82.7   |
| D . . . | 26.5                          | 40.2             | 8.6 | 17.9 | 93.2   | 5.3                           | 45.4             | 13.4 | 9.4  | 73.5   |
| E . . . | 30.5                          | 41.5             | 6.0 | 18.5 | 96.5   | 5.6                           | 46.3             | 22.2 | 10.1 | 84.2   |

removed and the parts analysed. The results are shown in Table VIII. In all cases the four elements constitute over 90 per cent. of the total ash of the kernel. The results for the pulp are more variable, and here the four elements constitute between 70 and 90 per cent. of the total ash. The possible significance of the high preponderance of these four elements in the ash of the kernel will be discussed later.

## VIII. DISCUSSION

In the following argument two assumptions are made. The first is that very little, if any, movement of calcium takes place in the phloem. This assumption is based on the findings for the cotton plant of Mason and Maskell (1931) and Mason and Phillis (1936). They found repeatedly that there was no evidence of calcium mobility in the phloem. The second assumption is that the relative proportions of the mineral elements in the transpiration stream is tolerably constant, at least over the period with which we are here concerned, namely 170 days.<sup>1</sup> This assumption appears to be supported by the following consideration. Suppose  $p$  is the amount of water in grammes entering the pulp from the transpiration stream in period  $t$ , and suppose the total amount of water  $X$  entering the fruit during the same period contains  $x$  grammes of calcium. If it is assumed that no actual transpiration takes place from the pulp, and this is

<sup>1</sup> During the wet season in Trinidad when soil water is never a limiting factor.

likely to be a small amount if not negligible, then the amount of calcium present in the pulp per 100 gm. of water will be  $\frac{100px}{pX} = \frac{100x}{X}$ . If, therefore, the value of this expression is constant over a period of time, it would be strong evidence that the concentration of calcium in the transpiration stream is con-

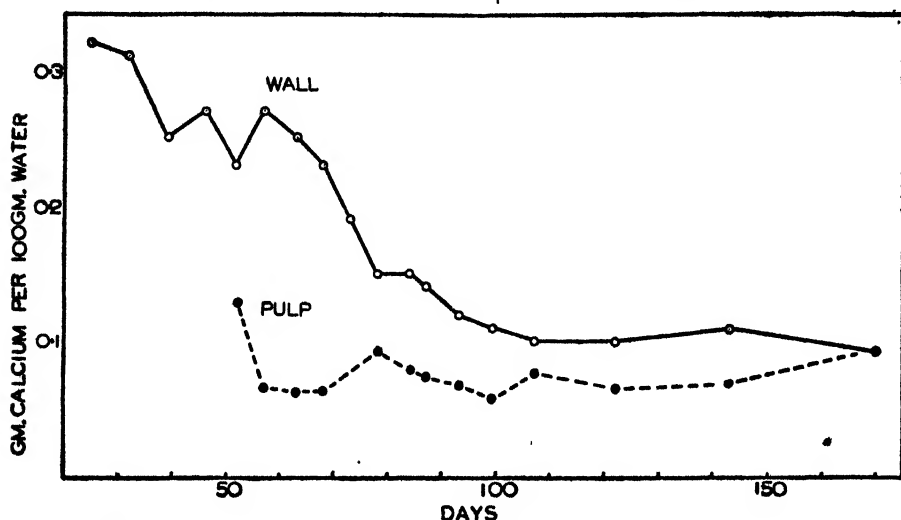


FIG. 4. The amount of calcium, gm. per 100 gm. water, in the wall and pulp at successive stages of development.

stant during the same period. Reference to Fig. 4 indicates that the amount of calcium per 100 gm. of water in the pulp is practically constant over the period for which data are available, viz. from 52 to 170 days. In the same figure are plotted similar data for the wall. In this case there is a continuous drop of calcium per 100 gm. water from the youngest stages until about 100 days, when the values remain constant. The shape of this curve indicates that the rate of transpiration from the wall is highest in the young fruit and falls as development proceeds.

The fruit may be regarded as a 'sink', *par excellence*, since re-export of substances from it is unlikely to take place under normal conditions, whereas re-exportation of concentrated and elaborated substances takes place from the leaf as a part of the normal function. It will be recalled that the relative (logarithmic) uptakes of potassium, phosphorus, and calcium are proportional to the relative dry weight uptake in the wall and pulp during the first 50 days of development. This may be an indication that during this period transport of mineral substances is chiefly via the xylem. A comparison of the ratio of potassium, phosphorus, or magnesium to calcium at different stages of development should give an indication whether the minerals are moving into the fruit mainly by the transpiration stream or in an elaborated form via the phloem. Relatively little change in the ratio of a particular element to calcium

with time would indicate that the demands for this particular element are met solely by the amount brought in the transpiration stream and that no 'demand' gradient for that element is set up via the phloem. If this assumption is correct it would be expected that the ratios in the wall would be more constant than those in the pulp, since in the former there is much less metabolic activity

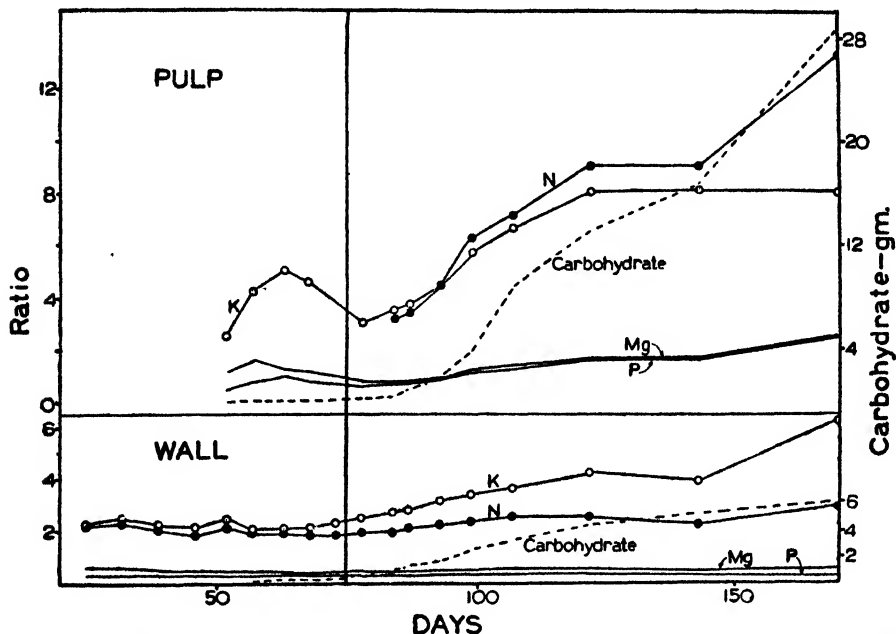


FIG. 5. The ratios of nitrogen, phosphorus, potassium, and magnesium to calcium in the wall and pulp at successive ages. The amounts of carbohydrate in the wall and the pulp at successive ages are also shown. For explanation see text.

and little demand for elaborated materials. It will be noticed in Fig. 5 that the ratios of phosphorus and magnesium to calcium in the wall are remarkably constant throughout the development of the fruit, which suggests on the above assumption that these minerals reach the wall almost entirely via the transpiration stream and that no gradient via the phloem is established on their account. The ratio of total nitrogen to calcium in the wall, on the other hand, while remaining practically constant up to about 75 days, thereafter shows a steady rise which is taken to indicate the import of nitrogen via the phloem. The ratio of potassium to calcium shows a somewhat similar trend. It is practically constant up to 75 days and afterwards increases until maturity. Potassium is probably directly concerned with carbohydrate transformations, and the rise in the ratio after 75 days suggests that potassium is imported into the wall via the phloem.

In the pulp the ratio of any element to calcium shows a sustained increase after 75 days. Before this time—figures are only available from 52 days onwards—most of the ratios show a rise and then a fall, this being most marked in



the case of potassium. This rise is due to the fact that the increase in calcium in the pulp is very slight at first, and since the amount of calcium is small in these samples and not very much material was available for analysis there may be a comparatively large error in these determinations which would affect the magnitude of the ratio very markedly. It is therefore assumed that in the pulp also the ratio of any element to calcium is fairly constant up to 75 days. From this time onwards there is a continuous increase in the ratios of potassium and nitrogen to calcium, and it is interesting to note that the lines for potassium and nitrogen are parallel, suggesting that they are imported at similar rates. In the same figure the absolute amounts of labile carbohydrate plus fat at different ages are shown, to give some idea of the rates of import of these substances, or their precursors, which doubtless takes place via the phloem. The phosphorus/calcium ratio and the magnesium/calcium ratio both show upward trends, the phosphorus and magnesium lines, however, being less steep than those of potassium or nitrogen. These slopes are in accordance with what would be predicted for the relative utilization of the various elements.

It is suggested therefore that up to 50 days both the wall and pulp receive their mineral elements chiefly by way of the transpiration stream and at that time very little demand for elaborated mineral substances has arisen, hence only a slight or negligible gradient for these substances has been established in the phloem. Between 50 and 60 days the first division of the fertilized embryo takes place, but until about 75 days the demand for elaborated substances is not very great and transport of minerals is still mainly via the xylem, hence the element/calcium ratio remains practically constant. After this time, however, the import of elaborated materials via the phloem commences actively and the value of the ratio increases steadily. It is not supposed, however, that the xylem transport ceases after 75 days; this undoubtedly continues in increasing absolute amounts in accordance with increasing surface of pod and therefore presumably increasing net transpiration per pod. It is suggested that the development of the pod is now not so dependent on the minerals brought by the transpiration stream, and slight deficits set up in this channel would not tend to check growth as it would in the earlier stages, since minerals are now available from other sources. It was previously pointed out that a cacao fruit is susceptible to wilt up to a period of 75 days, a time when according to the above theory the fruit relies mainly on the xylem for its supply of mineral elements; hence it would tend to be very sensitive to water strain or competition for minerals, either condition being likely to cause wilting. When, however, additional sources of supply via the phloem are available the second condition apparently becomes less critical.

The fact that in the kernel of the bean the total ash consists mainly of potassium, calcium, magnesium, and phosphorus might at first sight appear to be due to some form of selective mechanism, but it could also be interpreted as supporting the idea that during the later stages of development the phloem is the most important path of transport. If much of the mineral elements had reached the kernel via the transpiration stream one would expect to find a

larger amount of superfluous elements which would inevitably be carried up. This is not the case, and moreover the amount of calcium present is small. Hence it may be concluded that most of the mineral substance present in the kernel arrived as a result of gradients set up via the phloem. The rest of the pulp (white tissue plus testa), on the other hand, shows a greater percentage of calcium in the ash and a larger amount of unidentified elements, which suggests that the transpiration stream here played a more important part than in the case of the kernel. It is also of interest to note that for the testa the average magnesium/calcium ratio of the five samples (Table VII) is 0.56 and the phosphorus/calcium ratio is 0.23; these are not very far different from the corresponding ratios in the wall throughout development, suggesting that these were approximately the proportions of these elements in the transpiration stream at the time of the experiment.

The cacao fruit appears to be unusual in having a comparatively long period (75 days out of a total development time of 170 days) in which it is susceptible to physiological wilt. In the light of the foregoing investigation it appears that this is due to the fact that in this early period the fruit is largely dependent on xylem transport for its supply of mineral elements and water. This in turn appears to depend on the fact that embryo development begins rather late (between 50 and 60 days), so that 'demand' gradients for elaborated mineral substances are not set up in the phloem until a fairly late stage. In other fruit trees, such as apples, pears, &c., the 'danger period' during which fruits are liable to be lost by physiological wilt is a relatively shorter part of the total development time; this is apparently due to the fact that the development of the fertilized ovum begins earlier. A review of the available literature, however, shows that our knowledge of the time-relationships of embryo development of various fruits is very fragmentary so that it is not possible to enlarge upon this theme at the present time.

## IX. SUMMARY

1. The changes in mineral content (total ash, nitrogen, phosphorus, potassium, calcium, and magnesium) during the development of a cacao fruit have been studied.

2. The results are expressed on a dry-weight, on a residual dry-weight, and on an absolute basis (amount per fruit). There is a large increase in phosphorus content of the pulp during the pre-ripening and ripening stages, and there appears to be an actual loss of phosphorus from the wall during the latter stage.

3. The relative (logarithmic) rates of uptake of nitrogen, phosphorus, potassium, and calcium by the wall are constant and equal during the period from 25 to 57 days. This rate is also equal to that for dry matter increase over the same period. After 57 days the rates become less but are maintained up to 107 days. In this second phase the relative rates of uptake of the individual elements are no longer equal to one another, potassium alone maintaining a

rate equal to that of the dry matter increase. Magnesium appears to behave slightly differently from the other elements. The relative rates of uptake of nitrogen, phosphorus, potassium, and calcium in the pulp are equal to one another and to the relative rates of uptake in the wall during the first phase, but these rates are maintained in the pulp up to 107 days.

4. It was found that in the form of their oxides the four elements, potassium, calcium, magnesium, and phosphorus, constitute over 90 per cent. of the total ash of the kernel of a ripe cacao bean.

5. By assuming that calcium does not move in the phloem and that the relative proportions of the mineral elements in the transpiration stream are constant, it is concluded that, during the first 75 days of development of the fruit, mineral substances are imported mainly via the xylem. Hence during this period the young fruit would tend to be sensitive to water strain or competition in such substances, either condition being likely to cause wilting. Previous evidence had indicated a period of 75 days as a critical period in the development of the fruit.

6. It is suggested that the long critical period of the cacao fruit (75 days) is due to the late development of the fertilized ovum.

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# Protoplasmic Growth in the Hypanthium of *Oenothera acaulis* during Cell Elongation

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With two Figures in the Text

## I. INTRODUCTION

EVER since the discovery of phytohormones research work on the growth of plant cells has been confined exclusively to the direct physiological effects of auxins and hetero-auxin on cell growth. Only in the last few years has attention also been drawn to other processes associated with this growth. Thus recent papers by Schoch-Bodmer (1939), Beck (1941), and Burstroem (1942) have been devoted to the osmotic conditions during cell growth. Heyn (1940) summarized our knowledge of the alterations of the plasticity and elasticity of elongating cell walls. Just as interesting are the alterations of the submicroscopic structure of cell walls in the course of their elongation; Bonner (1936) and Frey-Wyssling (1938, 1941) have submitted these changes to close investigation.

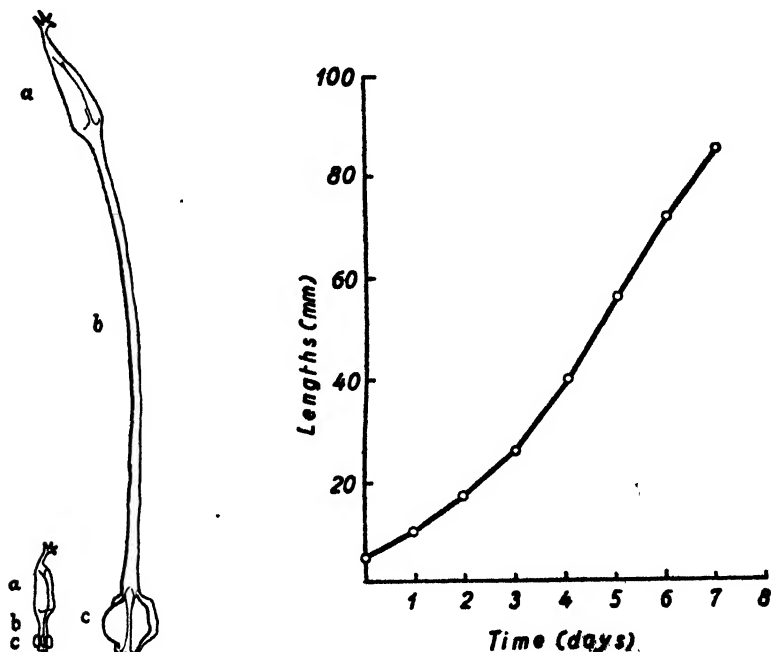
With regard to the physiological behaviour of protoplasm it has hitherto been the generally accepted opinion that no growth of the protoplasm takes place during cell elongation. No important function was attributed to the protoplasm in the course of this elongation, as can be gathered from the manuals and research work of Pfeffer (1897), Jost (1913), Sierp (1928), Priestley (1929), Went (1931), Miller (1938), Boysen-Jensen (1939), and Beck (1941).

In the course of two investigations (Frey-Wyssling and Blank, 1940, Blank and Frey-Wyssling, 1940, 1941), however, both an increase of the volume of the protoplasm and an enlargement of its protein framework were observed during cell elongation. These investigations were made with filaments of *Secale cereale* and the coleoptiles of *Zea Mays*. It seemed particularly interesting to investigate the behaviour of the protoplasm in some organ of a dicotyledonous plant with a view to ascertaining whether in this case also a growth of the protoplasm was to be observed. If so one could assume that the growth of the protoplasm was a general phenomenon in the course of cell elongation.

The hypanthium of *Oenothera acaulis* Cav., a part of the floral axis between the ovary and petals, was chosen for investigation. The test seedlings were grown separately in pots placed in a hothouse at a temperature of 18° to 20° C.

## II. LONGITUDINAL GROWTH OF THE HYPANTHIUM

The hypanthium is often found in the Onagraceae. This portion of the floral axis (Fig. 1) is known to grow very rapidly in length, although growth



FIGS. 1 and 2. *Oenothera acaulis*. Fig. 1. Bud and elongated floral axis showing the greatly elongated hypanthium, b. Fig. 2. Growth curve of hypanthium.

begins only a few days before the bud opens. Despite this the hypanthium of several species of *Oenothera* can attain 15 cm. in length within a few days.

In our experiments the measurements of the growth of *Oenothera acaulis* Cav. showed a growth curve (Fig. 2) slightly different from that found by Weinland (1941).

In most cases the buds opened on the evening of the seventh day, when the hypanthium had reached a length of 8.5 cm.

Thanks to the courtesy of Prof. J. Schwemmler we were able to experiment with the same seeds as were used by Mrs. Weinland. The variation in the rate of growth must thus be ascribed to varying experimental conditions.

Weinland (1941) also investigated in detail the growth of the hypanthium of

*O. acaulis* from the cytological point of view. She ascertained among other things that from 4 mm. onwards the longitudinal growth of the hypanthium depended exclusively on cell elongation. In *O. longiflora* and *O. missouriensis* actual elongation growth sets in only later.

In order to examine hypanthia showing purely elongation growth, only those in stages of development between 5 and 80 mm. long were used for our experiments, in accordance with Weinland's data. Although hypanthia go on growing after the buds have opened the experiments were stopped at a length of 80 mm., for according to Schumacher's investigations (1932) a back-flow of the nitrogen fractions from the petals may set in when the buds open.

### III. ALTERATIONS IN FRESH AND DRY WEIGHTS

The fresh weights were ascertained immediately after the hypanthia were cut from the experimental plants and after the styles had been removed from the hypanthia. To ascertain the dry weight the hypanthia were prepared in similar fashion and then dried in a thermostat for 3 hours at a temperature of 105° C. The results of these processes are shown in Table I.

TABLE I

*Fresh and Dry Weight of a Hypanthium in various Stages of Development*

| Length (mm.). | Fresh weight (mg.). | Dry weight (mg.). |
|---------------|---------------------|-------------------|
| 5             | 7.8                 | 1.9               |
| 20            | 30.0                | 3.5               |
| 30            | 46.0                | 6.4               |
| 40            | 65.6                | 7.6               |
| 50            | 89.3                | 8.9               |
| 60            | 112.5               | 10.8              |
| 70            | 130.7               | 13.2              |
| 80            | 159.1               | 15.4              |

The weights shown in Table I give a picture of the active metabolism during the cell elongation of this receptacle; both fresh and dry weight increase steadily. The fresh weight increased 20.4 times and the dry weight 8.1 times.

### IV. ALTERATIONS IN THE NITROGEN CONTENT

After cutting off hypanthia and removing the style the treatment of the experimental material was carried out immediately. To determine the total nitrogen content the hypanthia were at once digested in the Kjeldahl apparatus; to ascertain the amount of protein nitrogen and of non-protein nitrogen they were ground and the treatment continued immediately in order to avoid experimental errors caused by decomposition of the material. The results of these analyses constitute mean values, as in the case of both the fresh and the dry weights several hypanthia were analysed. All analyses were duplicated.

In all the experiments the micro-Kjeldahl method was used to determine the nitrogen. The organic substance was placed in retorts of 150 c.c. and digested

with 3 c.c. of concentrated sulphuric acid by placing them over a Bunsen flame for  $3\frac{1}{2}$  hours. A mixture consisting of seven parts sulphate of potassium and one part selenium was used as catalyst, since it was found in the experiments of Blackman and Templeman (1940) that when selenium was used as catalyst for the digestion of organic material the average nitrogen values were 6.5 per cent. higher than with copper sulphate. For the distillation of the sulphate of ammonia resulting from the digestion, the distillation apparatus by Parnas and Wagner as described by Pregl (1935) was employed. For the removal of the ammonia the digestion liquid was diluted with distilled water and then mixed with 18 c.c. of 30 per cent. sodium hydrate. As receiver *N/70* sulphuric acid to which three drops of methyl red were added as indicator, was used. When distillation was complete titration was effected with *N/70* sodium hydrate.

The separation of the protein nitrogen from the non-protein nitrogen was carried out with tannic acid. The hypanthia were ground after the addition of a few drops of toluol, then 10 c.c. of boiling 4 per cent. solution of tannic acid and 2 c.c. of 0.1 per cent. sulphuric acid were added. In each case coagulation set in at once. After 15 minutes 10 c.c., and after another 15 minutes a further 10 c.c. of pure alcohol were added. The mixture was filtered through a hardened filter (Schleicher and Schüll, No. 602) and rinsed with distilled water until the filtrate, received in a graduated flask, measured exactly 100 c.c. On the one hand exactly 50 c.c. of the filtrate and on the other the residue in the filter were examined according to the micro-Kjeldahl method. The nitrogen content of the residue was designated protein and that of the entire filtered material non-protein nitrogen.

In the first instance the alterations in the total nitrogen content of the hypanthium during elongation between 5 and 80 mm. was investigated. The following values were determined (Table II):

TABLE II

*Average Content of Total Nitrogen, Protein Nitrogen, and Non-protein Nitrogen of a Hypanthium in Various Stages of Development*

| Length (mm.). | Total nitrogen (γ). | Protein nitrogen (γ). | Non-protein nitrogen (γ). |
|---------------|---------------------|-----------------------|---------------------------|
| 5             | 148                 | 74                    | 72                        |
| 19            | 320                 | —                     | —                         |
| 20            | —                   | 154                   | 159                       |
| 31            | 390                 | —                     | —                         |
| 40            | 420                 | —                     | —                         |
| 45            | —                   | 210                   | 227                       |
| 50            | 445                 | —                     | —                         |
| 60            | 495                 | —                     | —                         |
| 70            | 550                 | —                     | —                         |
| 80            | 586                 | 286                   | 297                       |

The marked increase of all the three nitrogen fractions during cell elongation led us to repeat the series of experiments with an even larger amount of

test material in order to render the analyses statistically reliable and eliminate coincidence results. The results obtained were the following (Table III):

TABLE III

*Average Content of Total Nitrogen, Protein Nitrogen, and Non-protein Nitrogen of Hypanthia 5 mm. and 80 mm. in Length*

|                    | Total nitrogen (γ). | Protein nitrogen (γ). | Non-protein nitrogen (γ). |
|--------------------|---------------------|-----------------------|---------------------------|
| Hypanthium, 5 mm.  | 151                 | 71                    | 73                        |
|                    | 146                 | 74                    | 75                        |
|                    | 143                 | 79                    | 71                        |
|                    | 148                 | 70                    | 73                        |
|                    | 150                 | 78                    | 70                        |
|                    | 151                 | 71                    | 70                        |
|                    | 149                 | 73                    | 71                        |
|                    | 148.3 ± 1.1         | 73.9 ± 1.3            | 71.9 ± 1.3                |
| Hypanthium, 80 mm. | 593                 | 290                   | 295                       |
|                    | 582                 | 280                   | 301                       |
|                    | 605                 | 285                   | 290                       |
|                    | 610                 | 283                   | 297                       |
|                    | 602                 | 294                   | 300                       |
|                    | 587                 | 278                   | 305                       |
|                    | 598                 | 286                   | 298                       |
|                    | 591                 | 285                   | 297                       |
|                    | 605                 | 289                   | 302                       |
|                    | 589                 | 290                   | 299                       |
|                    | 586.2 ± 4.2         | 286 ± 1.5             | 298.6 ± 1.3               |

The results given in Table II were thus confirmed by subsequent analyses. Tables II and III show that the total nitrogen content of a hypanthium increases during cell elongation. The protein nitrogen and the non-protein nitrogen display a similar change.

The proportion between protein and non-protein nitrogen remains almost stable throughout the cell elongation. In hypanthia of 5 mm. length the content of protein nitrogen is a little greater, while in those of 80 mm. the non-protein nitrogen predominates to a small extent. Similar observations had been made in the course of experiments with rye filaments and coleoptiles of maize. In these filaments, which grow very rapidly, the amount of non-protein nitrogen remained constant. During the investigation of maize coleoptiles, on the other hand, the content of non-protein nitrogen increased quite noticeably.

## V. DISCUSSION

The number of cells remained constant during the cell elongation of the hypanthia examined. If all the hypanthium cells grew by the same amount, their length between the first and the last phase of development (5 and 80 mm.) increased 16 times. The fresh weight increased in this period to 20.4



times and the dry weight to 8.1 times the original. The total nitrogen, as well as the protein and non-protein nitrogen, quadrupled their original amount during the same period. With the exception of the fresh weight, which increased even a little more than the cell length, neither the increase of the dry weight nor that of the nitrogen fractions examined kept pace with the longitudinal growth. Thus the content of the cells was decreased in the course of elongation.

This change during longitudinal growth had already been observed during the experiments with rye filaments and coleoptiles of maize. The nitrogen content obviously decreases proportionally with increasing age and growth. Kocher (1941) came to the same conclusions in his investigations of the nitrogen content of leaves of *Melandrium album*.

The selection of the right relative magnitude is of the greatest importance when changes in the amount of a substance are studied during the growth of the plant. Comparison with the fresh and dry weights, which are themselves variable, would indicate a distinct decrease in the nitrogen content of the hypanthium cells during cell elongation, for the fresh and dry weights increased faster during elongation than did the nitrogen content. Such relative magnitudes may easily lead to wrong conclusions when processes connected with the growth of plants are studied; this is proved by the fact that, in relation to the whole hypanthium, all analyses resulted in an increase of the nitrogen fractions during cell elongation. As the number of cells remained constant during elongation, the content of the cells must, obviously, also have undergone an increase of all the three nitrogen fractions. During the examination of the maize coleoptiles it was thus possible, by combined cytological and microchemical investigations, to follow the increase of the nitrogen content.

The use of a physiologically-irreproachable reference magnitude, such as cells, parts of organs, organs or entire plants, appears to us to be the best basis for the proper judgement of physiological processes in a plant.

On the basis of cytological and microchemical findings, the reference works referred to in the introduction contained indications of the protoplasm growth during cell elongation. The results of the nitrogen analyses of hypanthia during the process of elongation again point to protoplasmic growth during cell elongation, for the polypeptides which, according to Frey-Wyssling (1938), form the framework of protoplasm, probably make up the larger part of the protein nitrogen. In the course of elongation, however, the proteins show an increase to four times the original amount.

On the basis of cytological observations alone protoplasmic growth might be traced back to the time of soaking.

The results of our analyses show, however, that the protein frame of the protoplasm increased during the process of elongation. There is, moreover, good reason to believe that also the lipoids, phosphatides, and nucleic acids built into the framework of the protoplasm undergo an increase during cell elongation. This means that the growth of the protoplasm is of an organic nature.

The entire process of cell elongation is quite different, as far as the behaviour of the protoplasm is concerned, from what has hitherto been assumed by most authors. Even if the protoplasm undergoes a certain 'dilution' during cell elongation it can be assumed, quite generally, on the basis of the present investigations of three different plant organs (filaments, coleoptiles, hypanthia), that protoplasmic growth occurs. In future studies on the highly complicated mechanism of cell elongation this fact should not be overlooked.

## VI. SUMMARY

The longitudinal growth of the hypanthium of *Oenothera acaulis* is extremely rapid. In the course of 6 to 7 days an increase from a length of 5 mm. to that of 80 mm. takes place by cell elongation. The length of the cells is multiplied on an average 16 times.

The substance changes in the hypanthium between the first (5 mm.) and the last (80 mm.) stage of development examined are the following: the fresh weight increases to 20.4 and the dry weight to 8.1 times the original. The total nitrogen, protein nitrogen, and non-protein nitrogen content increases to about four-fold the original magnitude.

From the increase of the protein nitrogen, and on the basis of results of earlier cytological and microchemical investigations on filaments of *Secale cereale* and the coleoptiles of *Zea Mays*, the conclusion is drawn that the protoplasm grows during cell elongation, and attention is drawn to the importance of this protoplasmic growth in the mechanism of cell elongation which has still to be elucidated.

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# The Effect of Vitamin B<sub>1</sub> on the Utilization of Glucose by *Melanospora destruens* Shear.

BY

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With one Figure in the Text

## INTRODUCTORY

IN a series of papers (Asthana and Hawker, 1936; Hawker, 1936, 1938, 1939, 1939 *a*, 1942) it has been shown that: (1) *Melanospora destruens* requires an external supply of biotin, i.e. vitamin H (György et al., 1940) for mycelial growth, (2) growth is further increased by the addition of vitamin B<sub>1</sub> (aneurin of European, thiamin of American writers), (3) perithecia are not formed<sup>1</sup> in the absence of vitamin B<sub>1</sub>, (4) formation of perithecia is reduced or inhibited by high concentration of glucose or of certain other carbohydrates, and (5) the concentration of glucose optimal for fruiting is raised with increasing concentration of vitamin B<sub>1</sub>.

The present paper is concerned with the influence of the vitamin on the utilization of glucose by the fungus.

The experimental methods were largely those described in previous papers. Where special methods were used they will be referred to in the text.

## RATE OF UTILIZATION OF GLUCOSE

The rates of utilization of glucose in the presence of different amounts of vitamin B<sub>1</sub> were determined. *M. destruens* was grown at laboratory temperature in medicine bottles laid on their sides and containing 20 c.c. of liquid medium *A* (Asthana and Hawker, 1936) in which the concentration of glucose was increased to 2.0 or 5.0 per cent. and to which was added 1 gm., 4 gm., or 10 gm. aneurin per 100 c.c. Biotin was supplied in the form of lentil extract since sufficient pure biotin was not available. The addition of a small amount of vitamin B<sub>1</sub> (1  $\gamma$  per 100 c.c. medium as shown by the Phycomyces test, Schopfer and Jung (1937)) contained in the extract was unavoidable, hence the amount of the latter added was as low as possible, viz. 0.05 per cent. This had the disadvantage that the amount of biotin present was also small. This

<sup>1</sup> Previously this fungus was able to grow sparsely and to produce a few perithecia on a synthetic medium in the absence of growth substances (Asthana and Hawker, l.c.). This ability has been lost in culture (Hawker, 1939, 1939 *a*).

difficulty could not be avoided in the absence of an adequate supply of pure biotin, but fortunately this substance is active at very low concentration so that the amount supplied was adequate. Five sample flasks were chosen at random 4, 7, 11, and 15 days after inoculation. The residual glucose in the medium was estimated by Bertrand's method and the dry weight of the mycelium was determined. Table I gives the results of a typical experiment in which the original concentration of glucose in the medium was 2.0 per cent. Mycelial growth and rate of consumption of glucose were both increased by the addition of aneurin to the medium, but the increase in mycelial growth was not in proportion to the increased consumption of glucose. This is brought out in columns 2 and 3 of Table I which give mg. glucose used per mg. dry wt. in media containing 1  $\gamma$  aneurin per 100 c.c. (derived from the lentil extract) and 11  $\gamma$  per 100 c.c. The results are shown to be significant by Fisher's method (Fisher, 1930, p. 107).

TABLE I

Glucose (mg.) consumed per mg. dry  
wt. of mycelium

| Days after<br>inoculation. | 1 $\gamma$ aneurin<br>per 100 c.c. | 11 $\gamma$ aneurin<br>per 100 c.c. | <i>t</i> | Value of <i>P</i><br>when <i>n</i> = 8. |
|----------------------------|------------------------------------|-------------------------------------|----------|---|
| 4                          | 2.05                               | 6.34                                | 6.46     | < 0.01                                  |
| 7                          | 3.03                               | 4.67                                | 6.75     | < 0.01                                  |
| 11                         | 3.80                               | 4.85                                | 6.77     | < 0.01                                  |
| 15                         | 3.89                               | 5.14                                | 5.33     | < 0.01                                  |

Six similar experiments gave results of the same order. The effects of increased aneurin were less marked in additional experiments where the vitamin was given in the form of lentil extract, owing to the increased growth resulting from the additional biotin, carbohydrate, and nitrogen unavoidably added with the larger dose of extract. The results, however, were in general agreement with those obtained with the pure preparation.

Thus it is clear that vitamin B<sub>1</sub> causes a greater increase in the rate of glucose consumption than would be expected from a consideration of the increase in weight of mycelium. This might be accounted for by increased storage of some carbon compound in the mycelium, by the accumulation in the medium of carbon compounds, either in the form of growth substances such as inositol (which *M. destruens* is known to synthesize, Hawker 1939 *a*) or staling substances such as bicarbonates or organic acids, or most probably by increased respiration. These possibilities were investigated.

#### POSSIBLE STORAGE OF CARBON IN MYCELIUM

Storage of carbon in the mycelium could not account for the increased rate of sugar consumption since the weight of the extra sugar consumed in the presence of vitamin B<sub>1</sub> was actually greater than the dry weight of the mycelium. Nevertheless it was possible that some of the extra consumption

could be accounted for by storage. Direct estimation of the amount of carbohydrate, &c., in the mycelium was not attempted, but the amount of carbon compounds stored in the mycelium was shown to be negligible by the following method. Muslin was stretched over rings of cane of a size to fit loosely in Petri dishes of 9 cm. diameter. These were boiled to remove any toxic dressings from the muslin, sterilized by autoclaving and placed inside sterile Petri dishes which were then poured with 20 c.c. liquid medium. A small piece of an agar culture of *M. destruens* was placed in the centre of each piece of muslin. The fungus was thus supplied with culture medium, but could be lifted out with the muslin-covered rings when desired. Cultures of this type were set up in a modified medium *A* containing 2.0 per cent. glucose and 0.05 per cent. lentil extract and in the same with the addition of 10  $\gamma$  aneurin per 100 c.c. medium. Four days after inoculation a sample comprising one-third of the cultures was taken and the mycelial dry weights and the amounts of residual glucose in the media were determined. Of the remaining cultures half were left in the original medium and half were lifted out, rinsed in two changes of sterile water, and transferred to medium *A* without glucose but with 0.05 per cent. lentil extract. Three days later the dry weights of the mycelia and the amounts of glucose in the media were determined. The results given in Table II show that after transfer to a sugarless medium mycelial growth ceased, whereas similar cultures remaining in the original media continued to grow. Moreover there had been no diffusion of glucose into the sugarless medium from the mycelium. Thus the mycelium could not have contained any reserves of carbon in an available form.

TABLE II

| Medium.  | Days after inoc. | Dry wt. of mycelium (mg.). | Glucose in medium (%). |
|--|------------------|----------------------------|------------------------|
| <i>A</i> 2% glucose + 0.5% extract . . . . .   | 4                | 72                         | 1.210                  |
| " " " " + aneurin . . . . .  | 4                | 79                         | 0.934                  |
| " " " " . . . . .  | 7                | 106                        | 0.406                  |
| " " " " + aneurin . . . . .  | 7                | 146                        | 0.164                  |
| " " " " culture  |                  |                            |                        |
| transferred to <i>A</i> without glucose after 4 days . . . . .   | 7                | 70                         | 0.0                    |
| <i>A</i> 2% glucose + 0.5% extract + aneurin; culture transferred to <i>A</i> without glucose after 4 days . . . . . | 7                | 73                         | 0.0                    |

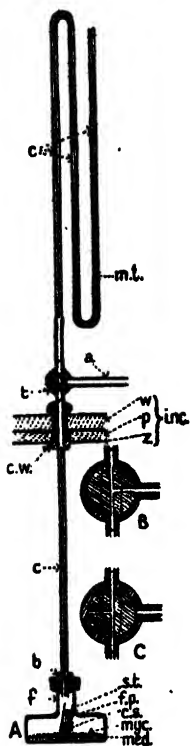
#### POSSIBLE ACCUMULATION IN THE MEDIUM OF METABOLIC PRODUCTS CONTAINING CARBON

Hawker (1939 *a*) has shown that *Nematospora gossypii*, which requires an external supply of inositol, is able to grow in mixed culture with *M. destruens* in the presence of biotin. Thus the latter fungus must be able to synthesize inositol. Growth of *N. gossypii*, however, was poor although an optimum amount of biotin was present, so that it is unlikely that the amount of inositol

produced by *Melanospora* is sufficient to account for more than a negligible amount of the glucose consumed.

There remains the possibility that a significant amount of carbon is present in the medium in the form of staling products, viz. bicarbonates or organic acids which are known to be formed by certain fungi (Pratt, 1924 a). *M. destruens*, however, is not a strongly 'staling' type of fungus. The mycelium fills the plate and there is no wavy margin to the colony such as indicates staling in *Fusarium fructigenum* (Brown, 1925). Moreover, the pH value of the medium did not alter greatly during the experiments, a change from 6.4 to 6.8 taking place in the first 7 days after inoculation, this change being independent of the initial concentration of sugar or vitamin B<sub>1</sub>.

Thus the greater consumption of sugar in the presence of aneurin cannot be explained by a greater accumulation of metabolic products containing carbon in the aneurin medium.



A, manometer attached to culture flask. c = capillary tubing, m.l. = manometer liquid, t = tap, a = arm of manometer, inc. = roof of incubator (w = wood, p = packing, z = zinc lining), c.w. = cotton wool, b = rubber bung, f = culture flask, s.t. = small glass tube, f.p. = folded strip of filter paper, c.s. = caustic soda solution, myc. = mycelium, med. = culture medium. B and C show tap in open and closed positions respectively.

#### EFFECT OF ANEURIN ON RATE OF RESPIRATION OF *M. destruens*

The measurement of respiration rate of *M. destruens* in the presence of different concentrations of lentil extract and later of pure vitamin B<sub>1</sub> was first undertaken by G. M. Brown (unpublished data) working in this laboratory. He used a modification of the apparatus described by Gregory and Sen (1937) and in preliminary experiments obtained an increase in the amount of carbon dioxide produced per unit dry weight of mycelium with increased concentration of vitamin B<sub>1</sub> in the medium. Thus in three experiments the respiratory index on a

medium containing 5 per cent. glucose and 0.1 per cent. lentil extract was increased by the addition of 10  $\gamma$  aneurin per 100 c.c. medium from 12.2 to 15.9, 14.6 to 18.3, 13.7 to 16.3 with 5-day-old cultures, and from 10.4 to 13.1, 12.8 to 18.3, and 14.6 to 15.3 with 6-day-old cultures. Unfortunately this work was interrupted by the outbreak of war. It was subsequently continued by the writer, who used the following manometric method.

Twelve manometers were constructed from glass capillary tubing (see Fig., p. 82). These were each fitted with a tap (B, C) so that the liquid in the manometer could be levelled. The part of the tube below the tap passed down into a lagged air-thermostat at 25.0° C. The fungus was grown in flat culture flasks each containing 20 c.c. of liquid medium. When measurements of respiration were to be made, a glass specimen tube of 5 mm. diameter containing 2 c.c. of a strong solution of caustic soda (80 per cent. saturation) was placed in each flask so that it remained upright supported against the neck of the flask (see Fig., p. 82). A folded scrap of filter-paper was allowed to dip into the caustic soda solution in order to increase the absorbing surface. The flasks were then sealed on to the end of the manometer tube by means of a rubber bung. When respiration rates on two media were to be compared five flasks (or occasionally three) of each medium were used, and two uninoculated flasks were employed as controls. These were arranged at random in the experiment chamber. Immediately after the flasks had been attached to the manometer and placed in the experiment chamber the taps were closed (as in C) for a few minutes to test the seals between flasks and manometers. Faulty seals, if any, were then tightened up and tested again. The taps were then all opened (as in B) for one hour to allow the temperature inside the flasks to reach that outside. The taps connected to the control flasks were then closed for a few minutes to determine whether a constant temperature had been reached, as shown by the manometer levels remaining unaltered. The period of one hour was almost invariably sufficient, but when necessary a longer preliminary period was given. All the taps were then closed and readings of the manometer level were taken at half-hourly intervals. Only those cultures showing a steady rate were considered in assessing the results.

The volume of oxygen (corrected for temperature and pressure) absorbed was calculated by the following formulae: Vol. O<sub>2</sub> absorbed =  $\frac{Kp}{1+at}$ .

$1+at = 1.092$  for constant temperature of 25° C.

$$p = \frac{P_0 - (V - \pi r^2 h/2)(P_0 - h)}{V},$$

where  $p$  = proportion of original pressure due to O<sub>2</sub> absorbed,  $P_0$  = height of manometer liquid for 1 atmosphere (13,251 mm.),  $V$  = vol. of gas in flask in cu. mm.,  $K$  for flask containing  $V_1$  of gas =  $V_1/P_0$ ,  $r$  = radius of manometer tube in mm.

Since the source of carbon in the medium was glucose it was assumed that the volume of oxygen taken in was equal to that of carbon dioxide produced, and the weight of carbon dioxide given off was calculated. Results were expressed as mg. carbon dioxide given off per mg. dry weight mycelium in one hour. A lowering of the respiratory quotient has been shown (Sinclair, 1933, 1933 a) with tissue cultures taken from pigeons suffering from lack of vitamin B<sub>1</sub>, but in the present investigation measurements of oxygen intake obtained by the manometric method were comparable with those of carbon dioxide



given off obtained by G. M. Brown, and thus the assumption that the respiratory quotient is approximately unity is justified. Since the cultures used were young and actively growing and showed no empty hyphae it was considered that dry weight of mycelium provided at least as good a basis for the estimation of respiratory activity as would protein content as suggested by Gregory and Sen (1937).

In the majority of experiments the rate of respiration on modified medium *A* containing 5.0 per cent. glucose and 0.05 per cent. lentil extract was compared with that on the same medium with the addition of 10  $\gamma$  aneurin per 100 c.c. medium. In a few instances the amounts of glucose, lentil extract, or aneurin were different and in one experiment the lentil extract was replaced by pure biotin. Experiments were replicated three to five times and over 100 cultures were used in the series. The cultures were incubated at 22° C. for 4 days after inoculation before measurements of respiration were made. The mycelium was removed from the flasks immediately after the completion of respiration measurements and dried to constant weight in a desiccator.

Table III shows the rate of respiration (expressed as mg. carbon dioxide given off per gramme dry weight of mycelium per hour) in the presence of 1  $\gamma$  aneurin per 100 c.c. medium (given in the form of lentil extract) and 11  $\gamma$  per 100 c.c. medium (10  $\gamma$  of which was added as pure vitamin B<sub>1</sub> and 1  $\gamma$  in the form of lentil extract) in 10 experiments. Other experiments in which the amounts of sugar or vitamin were altered were in general agreement, as was a single experiment where pure biotin replaced lentil extract. The figures given in columns 2 and 3 of Table III are the mean values for the 10 experiments and the differences between them were shown to be significant by Fisher's method (Fisher, 1930, p. 106).

TABLE III

*Respiration of M. destruens (mg. CO<sub>2</sub> per gm. dry wt. mycelium per hr.)*

|              | 1 $\gamma$ aneurin<br>per 100 c.c. | 11 $\gamma$ aneurin<br>per 100 c.c. | Difference. |
|--------------|------------------------------------|-------------------------------------|-------------|
| 1 . . . .    | 7.4                                | 8.0                                 | 0.6         |
| 2 . . . .    | 3.3                                | 10.1                                | 6.8         |
| 3 . . . .    | 9.9                                | 18.1                                | 8.2         |
| 4 . . . .    | 8.4                                | 10.5                                | 2.1         |
| 5 . . . .    | 12.5                               | 19.6                                | 7.1         |
| 6 . . . .    | 6.2                                | 16.8                                | 10.6        |
| 7 . . . .    | 5.2                                | 14.7                                | 9.5         |
| 8 . . . .    | 4.0                                | 9.9                                 | 5.9         |
| 9 . . . .    | 3.7                                | 5.8                                 | 2.1         |
| 10 . . . .   | 6.8                                | 13.0                                | 6.2         |
| Mean . . . . | 6.74                               | 12.65                               | 5.91        |

$$n = 9, t = 5.8645, P = 0.01$$

No direct comparison between the data of Table I and Table III is possible since the cultures referred to in Table I were grown at room temperature in a centrally heated laboratory while those referred to in Table III were incubated at a constant temperature of 22° C. as, owing to war conditions, room tempera-

ture was likely to be too low at night. Hence the stage of development of 4-day-old cultures in the two sets of experiments was not identical. It is clear, however, that the difference in respiration rate is of an order to account for the increased rate of consumption of glucose in the presence of high concentration of aneurin.

It has already been pointed out that *M. destruens* does not provide ideal material for testing the effect of aneurin on respiration since it requires an external supply of biotin which, at present, can only be supplied in the form of a crude extract which itself contains a small amount of aneurin. Accordingly a parallel series of experiments was undertaken with *Phycomyces nitens*, which is able to synthesize biotin but requires an external supply of aneurin (Schopper, 1934). This fungus was grown on a modified medium A, in which potassium nitrate was replaced by asparagin since it is unable to use the former. The results (Table IV) were in agreement with those obtained with *Melanospora*.

TABLE IV

*Respiration of Phycomyces nitens (mg. CO<sub>2</sub> per gm. dry wt. mycelium per hr.)*

|      |   | 1 $\gamma$ aneurin<br>per 100 c.c.<br>medium. | 11 $\gamma$ aneurin<br>per 100 c.c.<br>medium. | Difference. |
|------|---|---|--|-------------|
| 1    | . | 25.0  | 39.3   | 14.3        |
| 2    | . | 12.2  | 45.7   | 33.5        |
| 3    | . | 22.1  | 40.9   | 18.8        |
| 4    | . | 18.8  | 23.4   | 14.6        |
| 5    | . | 17.8  | 21.3   | 3.5         |
| 6    | . | 10.2  | 14.2   | 4.0         |
| 7    | . | 19.7  | 27.0   | 7.3         |
| 8    | . | 4.0   | 18.2   | 14.2        |
| 9    | . | 15.4  | 20.7   | 5.3         |
| 10   | . | 15.4  | 22.9   | 7.5         |
| Mean | . | 16.06   | 27.36  | 11.3        |

$$n = 9, t = 3.9291, P = 0.01$$

## DISCUSSION

The stimulatory effect of growth substances on respiration and fermentation of bacteria and fungi (including yeasts) has been noted by several investigators. Thus Amand (1904) noted that growth and fermentation of a species of yeast in a salt-sugar medium was increased in the presence of bacteria. This was probably due to the production of growth substances by the bacteria. Copping (1929) measured the respiration of certain strains of yeast (including baker's and brewer's yeast) and showed that bios (the chemical nature of which was then unknown) caused an increase in respiratory activity and fermentation. More recently Fardon and Ruddy (1937) and Norris and Ruddy (1937) described a factor produced by the action of ultra-violet light on yeast cells, which could be separated from the cells by filtering or centrifuging and which stimulated the respiration of yeast. A similar effect was obtained with bios

prepared by Narayanan's (1930) method and with marmite. Later Norris and Kreke (1937) claimed that the factors influencing growth, fermentation, and respiration of *Saccharomyces cerevisiae* were not the same but could be separated as three fractions from malt combings. Cook et al. (1938) also described a respiration-stimulating factor extracted from yeast which increased the oxygen uptake of living yeast cultures by 150–250 per cent. Pratt and Williams (1939) stated that pantothenic acid increased the respiration of two yeasts and that vitamin B<sub>1</sub> had a smaller effect. Dammann et al. (1938) reported that vitamin B<sub>1</sub> did not increase the weight of the mycelium of *Fusarium graminearum* (*Gibberella Saubinettii*) but accelerated fermentation of glucose. Schopper (1943 a) obtained a similar result with *Rhizopus suinus*, while Hills (1938) found that the oxygen intake by *Staphylococcus aureus* which was small in the absence of aneurin was greatly increased by the addition of an optimal dose of the vitamin.

The production of growth substances by certain parasitic fungi has been suggested as an explanation of increased respiration of the host plant in the presence of the parasite. Thus Yarwood (1934) showed that the rate of respiration of clover leaves was greater when these were parasitized by *Erysiphe polygoni* or *Uromyces fallens* and that this increase in respiration rate was maintained after the fungus had been killed by carbon disulphide. Allen and Goddard (1938), using wheat attacked by powdery mildew, attempted to measure respiration of host and parasite separately and concluded that the increase in respiration of diseased leaves was not due to the inclusion of fungal respiration in the estimation and postulated a stimulatory substance diffusing from the parasite. Hellings (1940) demonstrated an increased respiration of potato discs in the presence of extracts of *Gibberella Saubinettii*. The active substance was thermostable, non-volatile, insoluble in ether and chloroform, and adsorbed by activated coal or asbestos filter plates. Yeast extracts and crude peptone also increased respiration of potato, but preliminary experiments with pure aneurin gave mostly negative results.

A number of investigators [viz. Thompson, 1934; Sinclair, 1933, 1933 a; Lecoq and Duffau, 1938; Galvão and Pereira, 1937; and Joly, 1936] have reported a diminution of oxygen consumption and a lowering of the respiratory quotient in tissues from pigeons deprived of vitamin B<sub>1</sub> and a return to normal rates with the addition of the vitamin to the tissue cultures.

Thus there is considerable evidence for the influence of a growth substance, in some examples shown to be vitamin B<sub>1</sub>, on respiration of a number of plant and animal tissues. This is to be expected in view of the discovery that co-carboxylase is produced from aneurin, pyro-phosphoric acid and protein (Lohmann, 1937; Lohmann and Schuster, 1937), and Schopper (1943) in a recent book on plants and vitamins concludes that the chief role of aneurin is as a component of co-carboxylase. The synthesis of co-carboxylase from vitamin B<sub>1</sub> has been further described by Tauber (1937), Ochoa and Peters (1938), Lipschitz et al. (1938), while Silverman and Werkman (1939) have shown that this synthesis can be carried out by *Propionibacterium pentoraceum*.

If the views of Neuberg and Kerb (1912) and Kostytschev (1912), that one stage in respiration is the decarboxylation of pyruvic acid, are valid, the importance of vitamin B<sub>1</sub> in respiration becomes obvious. Thus Koser and Saunders (1938) in a review of accessory growth factors for bacteria and other micro-organisms suggest that 'most of them (growth substances) enter into the structure of enzymes or co-enzymes concerned with cell oxidation' and further state that the 'pyrophosphoric ester of thiamin (vitamin B<sub>1</sub>), thiamin diphosphate, functions as a co-carboxylase with a protein of yeast cells and in this enzyme system strongly promotes the decarboxylation of pyruvic acid—an important intermediate product in dissimilation of glucose'. Thus it is probable that in the earlier work cited above, where unidentified growth substances of the bios type stimulated respiration the active factor was vitamin B<sub>1</sub>. The stimulatory effect of pantothenic acid on yeast respiration described by Pratt and Williams (1939) may have been due to the effect of this growth substance on some other part of the respiratory cycle or to an indirect effect on growth and probably a consequently increased synthesis of aneurin. Where the pure vitamin was found to be less effective than the less pure extracts, lack of other growth substances may have been limiting.

Since phosphates are associated with vitamin B<sub>1</sub> in the production of co-carboxylase, it might be expected that phosphate concentration would also influence respiration. Harden (1932) discusses the role of phosphates in alcoholic fermentation and states that phosphate greatly increases the rate of fermentation by yeast juice and, to a lesser extent, that by zymine (dried yeast), but does not influence fermentation by fresh yeast. He suggests that the enzymes in the living cell are capable of breaking up hexose phosphates in the cell, thus releasing phosphate in sufficient quantity to prevent any increase in the external supply being effective, and that maceration destroys this power. It has previously been shown (Asthana and Hawker, 1936) that *M. destruens* is unable to produce perithecia in the absence of any external supply of phosphate, but that a reduction in the amount present to one-fifth that in medium A had no significant effect. No evidence was obtained in the present investigation to show that increased phosphate in the presence of high aneurin concentration would further increase respiration or the rate of removal of glucose from the medium, so that it may be concluded that the requirements, as with yeast, are low.

With *M. destruens* a high rate of respiration is correlated with increased production of perithecia. Thus the present paper shows that respiration is increased in the presence of a relatively high concentration of aneurin which has previously (Hawker, 1938 and 1939 a) been shown to increase fruiting. Moreover, more perithecia are produced on a sucrose medium than on a glucose one (Hawker, 1939), and respiration per unit dry weight of mycelium is greater on the former medium (Hawker, unpublished data). A somewhat similar effect was described by Brown (1925) with *Fusarium fructigenum*. High glucose concentration increased mycelial growth and depressed sporing, while high starch concentration gave less mycelium with the produc-

tion of numerous conidia. Nevertheless starch was used up more rapidly than glucose and Brown postulated a higher rate of respiration with the former. It is not possible to decide with the evidence available whether this correlation between spore production and a high respiration rate is due to the release of more chemical energy during intense respiration or to the accumulation of some other unidentified metabolic product during the process.

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### SUMMARY

The presence of 10  $\gamma$  aneurin (vitamin B<sub>1</sub>) per 100 c.c. medium increases the amount of glucose consumed per unit dry weight of mycelium by *M. destruens*.

The extra sugar consumed is not stored in the mycelium, and there is no large accumulation in the medium of metabolic products containing carbon such as inositol, bicarbonates, or organic acids.

The oxygen intake, and therefore presumably the amount of carbon respired, per unit dry weight of mycelium is increased by vitamin B<sub>1</sub> to an extent sufficient to account for the extra glucose consumed. A similar result was obtained with *P. nitens*.

Previous accounts of the effects of growth substances on respiration are summarized and discussed.

A correlation between high respiration rate and production of perithecia by *M. destruens* is shown.

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# Studies on the Biology and Pathogenicity of *Colletotrichum indicum*

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With six Figures in the Text

**I**N 1938 diseased cotyledons similar to those caused by anthracnose were noticed in a local variety of Asiatic cotton, *Gossypium arboreum* L., grown in the greenhouse at Chengtu, Szechuan Province, West China. Microscopic examination revealed that the causal fungus, though still a species of *Colletotrichum*, differs from the conidial stage of *Glomerella gossypii* (South.) Edg., the cause of common anthracnose, by its falcate-shaped conidia as well as the longer and more abundant setae. Later the same fungus was found in several parts of the cotton district in the same province. In August 1940 the disease became prevalent at Suinin, where about 2 per cent. of the loss in boll rot was estimated to be due to the fungus. Although this disease is not as common as the anthracnose and the bacterial angular spot, yet owing to the more rapid invasion of host tissue in both the seedling and boll stages it really causes greater damage than the two others.

In 1934 Dastur (1934) reported an anthracnose of cotton due to a new species of *Colletotrichum*, *C. indicum* Dast., from the Central Provinces, India, where it caused considerable damage. A comparison of his description with Iwadare the fungus found here in Szechuan indicates their apparent identity. (1937) noted an anthracnose disease on cotton boll in Manchuria, China, with falcate conidia. Hemmi (1937) considered that this fungus and another collected by T. Abe from Siduoka, Japan, were both identical with *C. indicum*. In Seymour's 'Host Index of the Fungi of North America' (1939), under *Gossypium hirsutum* L., two species of *Colletotrichum* are listed, *C. gossypii* and *C. falcatum* Went. Very likely the latter name refers to a fungus similar to *C. indicum* in the shape of its conidia. It seems possible that this new anthracnose may have long existed as a fairly common pathogen, at least in the cotton district of Asia, but until recently no effort has been made to distinguish it from the common anthracnose.

The symptoms, effect, and pathological histology of this new disease were studied in detail by Dastur (1934). Thomas (1939, 1940) and Ramakrishnan (1941) also reported their results of studies on the parasitism and physiology



of the causal fungus in Madras Province, India. The present paper deals with the morphology, physiology, parasitism, and life-history of the Chinese isolate of this fungus as studied since 1938.

### MORPHOLOGY AND TAXONOMY

The minute black dots, so abundantly produced on aged lesions of the diseased plants, are typical of the acervuli of a *Colletotrichum* or a *Vermicularia*. They are scattered, aggregated, or arranged in rings. On the basal stromata setae are produced and chiefly interspersed with the conidiophores, varying in number from 1 to 9 in each acervulus. The setae are dark brown in colour, long-pointed, and 1 to 3 septate. The conidiophores are hyaline, cylindrical, simple, and shorter than the conidia. Borne acrogenously and singly on the conidiophores, the conidia are falcate in shape, obtuse at the ends, homogeneous but often vacuolate in content, and measure  $16.5-27.5 \times 3.5 \mu$ .

When cultured on agar media, the acervuli, conidiophores, and conidia are larger in size and the setae are more abundant than those produced on the host in nature.

The morphology of the fungus agrees well with that of *Colletotrichum indicum* Dast. Although certain characters such as the width of conidia and conidiophores may differ somewhat, yet the differences are considered not beyond the range of variability that may exist between races within one species, especially under geographic influences. In Table I, measurements are given of the Chinese fungus on the host and on agar cultures, and also measurements of *C. indicum* as described by Dastur (1934). A comparison of these sets of measurements appears to justify the identity of the two fungi.

TABLE I

*Measurements of the Chinese isolate of Colletotrichum indicum in comparison with those described by Dastur from Central Provinces, India*

| Sub-stratum.                   | Diameter of acervuli ( $\mu$ ). |                  | Size of setae ( $\mu$ ).       |   | Size of conidia ( $\mu$ ).    |  | Size of conidiophores ( $\mu$ ). |  |
|--------------------------------|---------------------------------|------------------|--------------------------------|---|-------------------------------|--|----------------------------------|--|
|                                | Range.                          | Mean + S.E.      | Range.                         | Mean + S.E.                             | Range.                        | Mean + S.E.                            | Range.                           | Mean + S.E.                            |
| Cotton (Dastur)                | —                               | —                | $76.5-125.5$<br>×<br>$3.8-7.6$ | —                                       | $15-25$<br>×<br>$1.8-4.3$     | —                                      | $7.7-13.2$<br>×<br>$1.6-2.7$     | —                                      |
| Cotyledon of cotton (Authors)  | 27-124                          | $59.2 \pm 0.95$  | $40-216$<br>×<br>$3.0-7.7$     | $86.4 \pm 2.14$<br>×<br>$4.5 \pm 0.06$  | $16.5-27.5$<br>×<br>$3-5$     | $22.2 \pm 0.11$<br>×<br>$3.9 \pm 0.04$ | $5.5-15.1$<br>×<br>$1.9-4.1$     | $8.8 \pm 0.16$<br>×<br>$2.9 \pm 0.01$  |
| Potato-dextrose agar (Authors) | 36-384                          | $112.4 \pm 4.32$ | $69-399$<br>×<br>$3.3-9.1$     | $205.2 \pm 4.45$<br>×<br>$6.3 \pm 0.27$ | $19.3-33.0$<br>×<br>$2.8-4.4$ | $25.4 \pm 0.20$<br>×<br>$3.5 \pm 0.03$ | $8.3-34.4$<br>×<br>$1.9-3.3$     | $21.7 \pm 0.34$<br>×<br>$2.7 \pm 0.02$ |

As regards the generic position to which this fungus properly belongs, the question arises as to the criteria distinguishing between *Colletotrichum* and *Vermicularia*. The old idea that the latter genus is pycnidium- or sporo-

dochium-bearing is no longer tenable. Clements and Shear (1931) separated the two genera on the basis of the location of the setae, which are said to be marginal in *Colletotrichum* but scattered generally in *Vermicularia*. If this concept is accepted, then not only the fungus under consideration but also most species now classified as *Colletotrichum* should be transferred to the genus *Vermicularia*. Grove (1937) maintained that these two genera differ essentially in their mode of growth. In *Vermicularia* the setae are essential elements protruding above the matrix when hardly any mature spores have as yet arisen; whereas in *Colletotrichum* the setae may or may not be produced. Although such a difference does exist in this particular group of fungi, it seems inadequate to separate genera on the basis of such a character alone. Unless future research reveals a more tangible basis for their distinction it seems advisable simply to follow the view of Duke (1928) and consider the two generic names, *Colletotrichum* and *Vermicularia*, as synonymous.

### PHYSIOLOGY

**Germination of conidia.** Conidia of the fungus germinate readily in drops of water and on various agar media. At a favourable temperature germination may take place after 1 hour on potato-dextrose agar and within 3 hours in water. In most cases the conidia become uniseptate during germination. The germ tube, which measures  $3-4\ \mu$  in width, may arise at any point, though generally from the side of the conidium, near one end. Sometimes a second germ tube may be produced. The germ tube frequently forms a thick-walled and dark-coloured appressorium at its tip as a result of contact stimulus. Under suitable conditions for growth, the germ tube, arising either directly from the conidium or from the appressorium, branches profusely and develops into a mycelial mat after a short period of time.

**Temperature relations.** Conidial suspension made from young agar culture at standardized dilution was placed for germination on the surface of potato-dextrose agar in Petri dishes and was incubated in darkness at different temperatures. Triplicate plates were used in each test. After 2 hours of incubation, counts on the percentage of germination and measurements of the length of germ tubes were made at hourly intervals. The results obtained after 2 and 3 hours appear in Fig. 1. Under the conditions of these experiments the optimum temperature for germination was about  $32^{\circ}\text{C}$ . At  $16^{\circ}\text{C}$ . no germination occurred in 3 hours. In the range of  $20-36^{\circ}\text{C}$ . the percentages of germination increased rapidly after 2 hours, and all exceeded 80 per cent. within 3 hours. The elongation of germ tubes, however, proceeded most rapidly at  $28-32^{\circ}\text{C}$ . and declined on both sides of these figures.

The effect of temperature on the mycelial growth of the fungus was also determined by seeding inocula of uniform size on potato-dextrose agar in Petri dishes. The plates were then incubated, in four replications, at temperatures ranging from  $16^{\circ}$  to  $36^{\circ}\text{C}$ . Simultaneously, two strains of *Glomerella gossypii* were used for comparison. The data taken after 6 days are shown in Fig. 2.

Both *Colletotrichum indicum* and *G. gossypii* gave similar growth curves in that their optima lay somewhere around 28° C., with a gradual decline in growth at higher and lower temperatures. This finding deviates somewhat from that of

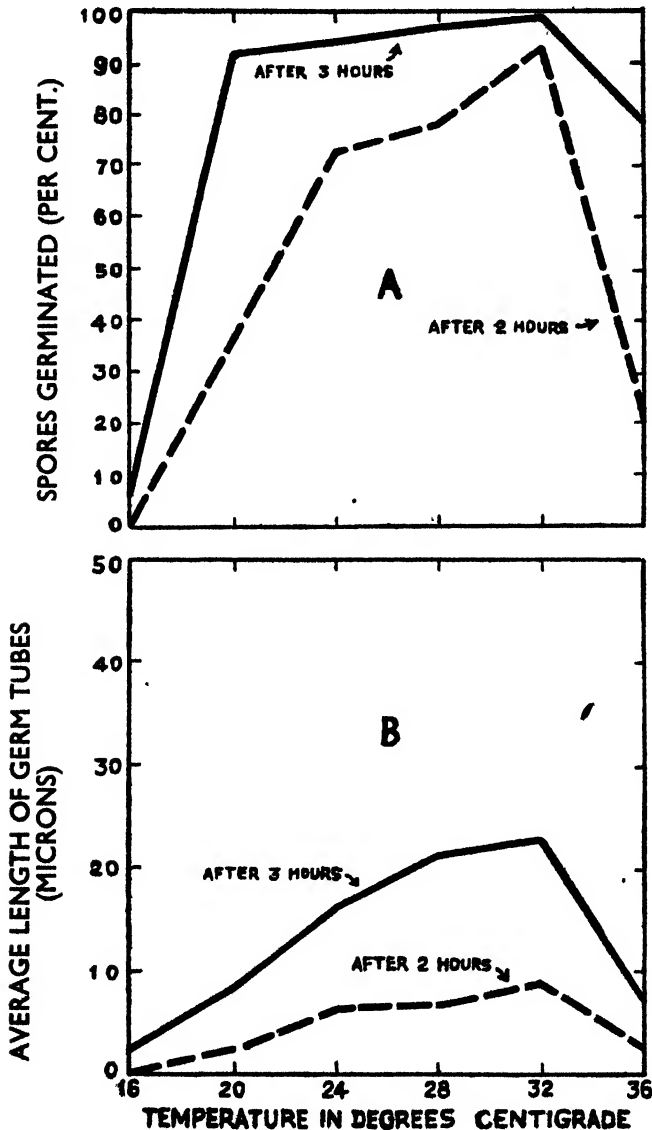


FIG. 1 A and B. The relation of temperature to the percentage germination (A) and to the length of the germ tube (B) of conidia of *Colletotrichum indicum* on potato-dextrose agar.

Thomas (1939), who reported the optimum for the growth of *C. indicum* to be near 32° C.

*Hydrogen-ion relations.* The effect of pH of the medium on the germination

of the conidia was studied in hanging drops in van Tieghem cells. The conidial suspension of standardized dilution was made in McIlvaine's standard buffer solutions of different pH, which were prepared by mixing 0.1 M citric acid and 0.2 M disodium phosphate according to Clark (1928). Since the germination took place much more slowly in such media than in water, counts

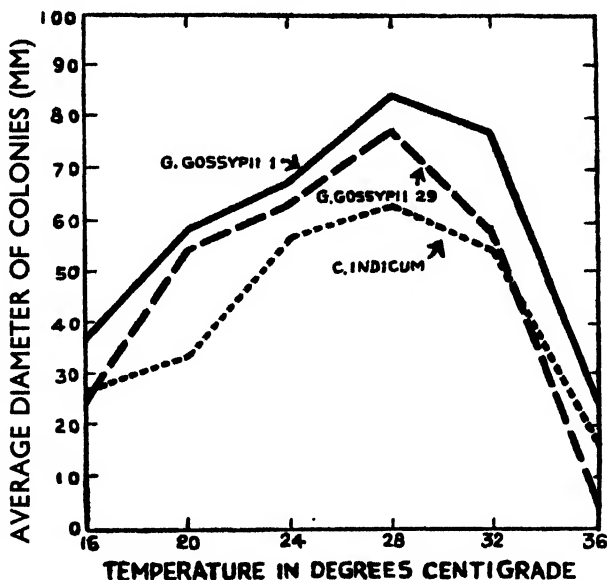


FIG. 2. The relation of temperature to the growth of *Colletotrichum indicum* and *Glomerella gossypii* on potato-dextrose agar after 6 days.

on the percentage and measurements of the length of germ tubes were made after 12 hours. Results as shown on Fig. 3 indicate that the optimum pH for the germination and the elongation of germ tubes is about 5.4.

Growth of *Colletotrichum indicum* on potato-dextrose agar in which the pH varied was determined on comparison with one strain of *Glomerella gossypii*. The pH of the medium was adjusted aseptically by the addition of certain amounts of normal solutions of NaOH and HCl after sterilization and immediately before use. The determination of pH was made colorimetrically. After seeding with inocula of uniform size, the plates in quadruplicate for each pH were incubated at 28° C. Readings were taken every other day. Mycelial growth of both fungi occurred throughout the range of pH tested, and both appeared to have similar and fairly wide range for their optimal growth. The results are averaged and shown in Fig. 4. Sporulation of *C. indicum* was best within the range of pH from 5.4 to 7.6.

**Cultural characteristics.** The fungus varies considerably in its rate of growth, characteristics of colony, and habit of sporulation on various agar media. Among the media tested, the Richard's and potato-dextrose were proved to be most suitable. The characters of the fungus as grown on five different media are briefly summarized in Table II.

**Resistance of conidia to desiccation.** The conidia of the fungus are very susceptible to desiccation. When they were smeared on glass slides either with their cirri or in water suspension and allowed to dry, they failed to germinate

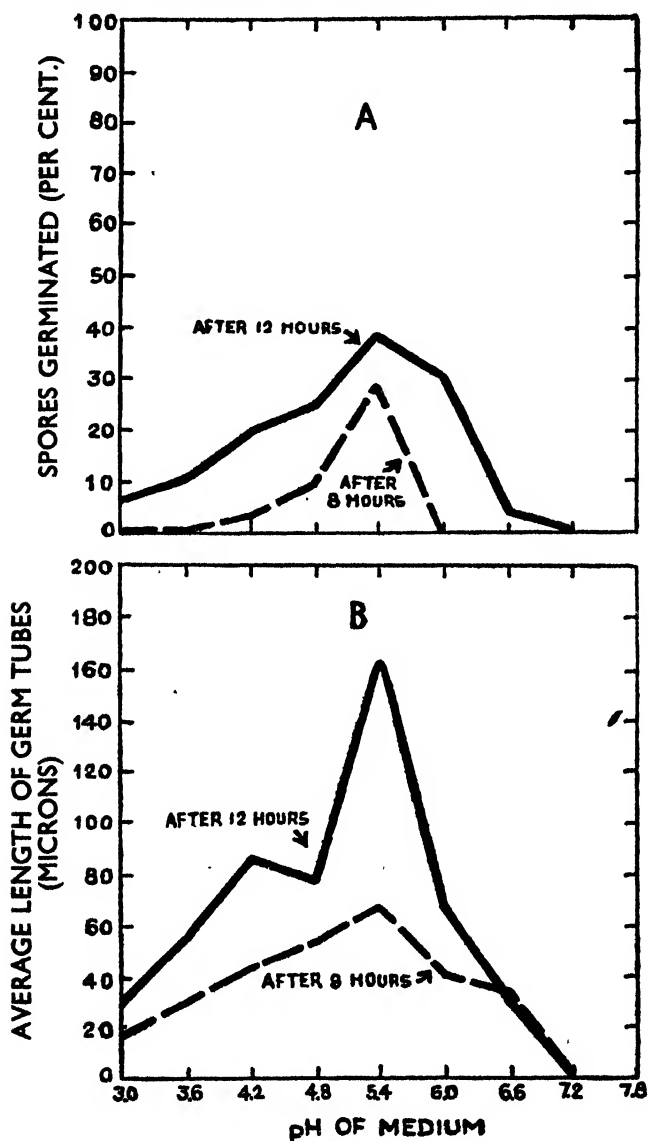


FIG. 3 A and B. The relation of H-ion concentration to the percentage germination (A) and to the length of the germ tubes (B) of the conidia of *Colletotrichum indicum* on potato-dextrose agar.

after being maintained at 28° C. for 24 hours. Germination tests were made at intervals by adding a drop of distilled water on each smear, and the results are recorded in Table III.

**Production of toxic substance.** Thomas (1939, 1940) reported that cotton seedlings placed in the filtrate of the fungus wilted, thus indicating the

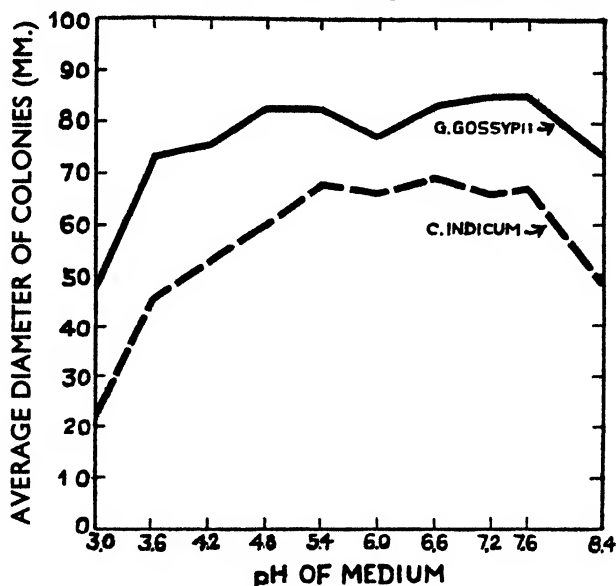


FIG. 4. The relation of H-ion concentration to the growth of *Colletotrichum indicum* and *Glomerella gossypii* on potato-dextrose agar after 6 days.

TABLE II

*Cultural Characteristics of Colletotrichum indicum on various Agar Media after 7 Days' Incubation at 28° C.*

| Agar medium.    | Average diameter of colony (mm.). | Mycelial growth.  |  | Edge of colony. | Production of acervuli.  |
|-----------------|-----------------------------------|---|--|-----------------|--|
|                 |                                   | Aerial.   | Submerged.                               |                 |  |
| Potato-dextrose | 71                                | Thick, white to pale olive-grey                                       | Light yellowish-olive to yellowish-olive | Entire          | Large, black, round to irregular masses of acervuli in the centre of colony.   |
| Cornmeal        | 61                                | Forming sparse tufts, moderately thick, pale olive-grey to pearl-grey | None                                     | Entire          | None.  |
| Oatmeal         | 68                                | Thick, pale smoke-grey  | None                                     | Entire          | Large, black, round masses of acervuli scattered on surface of medium.   |
| Pea             | 70                                | Creeping, thin white to pale olive-grey                               | None                                     | Entire          | None.  |
| Richard's       | 76                                | Moderately thick, pale ochraceous salmon                              | None                                     | Fimbriate       | Numerous black, medium to large, round to irregular masses of acervuli scattered on surface of medium, forming indistinct rings. |

The colours given are those of Ridgway (1912).

secretion of a toxin by the fungus. Although the production of toxic substance has been proved in certain wilt-causing organisms, such as *Bacterium solanacearum* E. F. Sm., *Phytophthora nicotinae* Breda de Haan, *Fusarium* spp., and others, this is not so with species of *Colletotrichum* or its related forms.

Thomas' results were not corroborated by ours. Ramakrishnan (1941), by histological studies, also failed to find any evidence that the fungus has a 'killing-in-advance' action.

TABLE III

*Effect of Desiccation on the Vitality of Conidia of Colletotrichum indicum*

| Medium in which the<br>conidia are dried. | Conidia (%) germinated after time stated. |    |    |    |    |         |
|---|---|----|----|----|----|---------|
|   | 0   | 2  | 6  | 12 | 24 | 48 hrs. |
| Water . . .                               | 46  | 44 | 35 | 16 | 0  | 0       |
| Cirri . . .                               | 83  | 9  | 22 | 17 | 0  | 0       |

The production of toxin was first tested in potato-broth culture of the fungus. The broth, after the organism had grown for 10 days, was filtered through a Mandler diatomaceous filter. Healthy seedlings of cotton were immersed in the filtrate. No indication of wilting was noticed after 5 days. Further tests were made with diseased plants, infected bolls and seedlings being macerated and extracted with distilled water. After filtration the extract failed to produce any ill effect on the cotton seedlings.

#### PATHOGENICITY

*Infection experiments.* Thomas (1939, 1940) and Ramakrishnan (1941) reported a reduction in the germination rate of cotton seeds artificially infected with *Colletotrichum indicum*. Our results obtained in 1938 and again in 1941 failed to confirm this. Seeds of both American and Chinese cotton were soaked in the conidial suspension of the fungus for 30 minutes, permitted to dry overnight, and then planted in germinating trays or in sterilized soil. Seeds soaked in distilled water and treated in a similar way served as a check. The number of seedlings emerging and those diseased were recorded after 2 weeks, when the infection could be readily recognized. As shown in Tables IV and V, though a high percentage of infected seedlings resulted from inoculation as against one or none in the controls, the difference between the inoculated lot and the control in either the rate of germination or in the rate of emergence from the soil was in every case rather small to be considered significant. This statement, however, does not exclude the possibility that under other conditions either the germination of the seeds may be reduced or the seedlings killed by the fungus before emergence. In the field if the seeds are badly infected, the mycelium of the fungus may penetrate deeply into the embryonic tissue and destroy their germinating power. The methods of inoculation described above and that used by Thomas (1939, 1940) do not seem to permit a deep penetration of the fungus into the seeds and appear unlikely to have such a serious effect. As regards pre-emergence killing, although the results presented in Table IV failed to indicate such effect, it is clear from Table V that the seeds used in our experiments were heavily infected by fungi other than *C. indicum*. Even in the control the total number of seedlings surviving was only slightly higher than that in the infected series. Therefore, most of the dead seedlings, though infected with *C. indicum* were not necessarily killed

by this fungus alone. If clean seeds had been used the result would no doubt have been different.

TABLE IV

*Inoculations of Cotton Seeds with Colletotrichum indicum in Sterilized Soil in 1938 and 1941. The numbers in brackets are percentages*

| Year. | Variety of cotton. | Treatment. | Seeds planted. | Seedlings emerged. | Seedlings infected with <i>C. indicum</i> . |
|-------|--------------------|------------|----------------|--------------------|---|
| 1938  | Trice              | Inoculated | 90             | 52 (57.7)          | 35 (67.3)                                   |
|       |                    | Control    | 30             | 15 (50.0)          | 0 (0)                                       |
|       | Local              | Inoculated | 90             | 14 (15.5)          | 14 (100)                                    |
|       |                    | Control    | 30             | 7 (23.3)           | 1 (14.3)                                    |
| 1941  | Delfos 531         | Inoculated | 240            | 158 (66.0)         | 97 (61.4)                                   |
|       |                    | Control    | 240            | 151 (62.9)         | 0 (0)                                       |
|       | Local              | Inoculated | 240            | 122 (50.8)         | 103 (84.4)                                  |
|       |                    | Control    | 240            | 178 (74.3)         | 0 (0)                                       |

TABLE V

*Inoculations of Cotton Seeds with Colletotrichum indicum in Germination Trays (1941). The numbers in brackets are percentages*

| Variety of cotton. | Treatment. | Seeds planted. | Seeds germinating. | Seedlings infected by <i>C. indicum</i> . | Seedlings killed by <i>C. indicum</i> . | Seedlings diseased of other causes. <sup>1</sup> | Total seedlings survived. |
|--------------------|------------|----------------|--------------------|---|---|--|---------------------------|
| Delfos 531         | Infected   | 216            | 152 (70.4)         | 60 (39.5)                                 | 44 (29.0)                               | 65 (42.8)  | 46 (30.3)                 |
|                    |            | 216            | 171 (79.2)         | 0 (0)                                     | 0 (0)                                   | 106 (61.6)                                       | 69 (40.4)                 |
|                    | Local      | 216            | 176 (81.5)         | 82 (46.6)                                 | 72 (41.0)                               | 65 (36.9)  | 39 (22.2)                 |
|                    |            | 216            | 181 (83.8)         | 0 (0)                                     | 0 (0)                                   | 117 (64.6)                                       | 64 (35.4)                 |

<sup>1</sup> Including those infected with *Glomerella gossypii*, *Fusarium* spp., and others of unknown causes.

At the seedling stage cotton is very susceptible to infection by *C. indicum*. By spraying with conidial suspension, lesions usually developed on cotyledons and young stems after 3 to 5 days (Fig. 5). On the cotyledons the initial lesions appeared as water-soaked, olivaceous brown, and more or less circular spots. Under humid conditions the spots soon enlarged and often extended to the stems through the petioles. The old lesions were brown at the centre and surrounded by a greenish border. On the stems the spots were elongate and reddish-brown. At a later stage the diseased parts became shrivelled and sunken, and eventually brought about the death of affected seedlings.

Mature leaves have never been found attacked by the fungus in nature, but the infection could be brought about by artificial inoculation, though successful only with the varieties of *Gossypium arboreum*. About 5 days after spraying with conidial suspension, small spots which were dark green in colour and water-soaked in appearance appeared on the leaves. With age the spots turned to greyish-brown in colour and became irregular in shape, with indistinct and dark brown margin and minute black dots scattered over the centre (Fig. 6). Old leaves appeared to be more resistant.

Detached bolls of both the local and American cotton were inoculated at different stages of maturity by placing a drop of conidial suspension on the uninjured surface and incubating in the moist chamber. Infection usually



became evident after 3 to 5 days. The infection manifested itself first as water-soaked, dark brown spots, which soon enlarged and turned to purplish-brown, with bristly, black acervuli produced at the centre. At that stage, if the humidity was not high enough to support further extension of the lesions, the central portion of the spots became dry, sunken, and black, with a brown

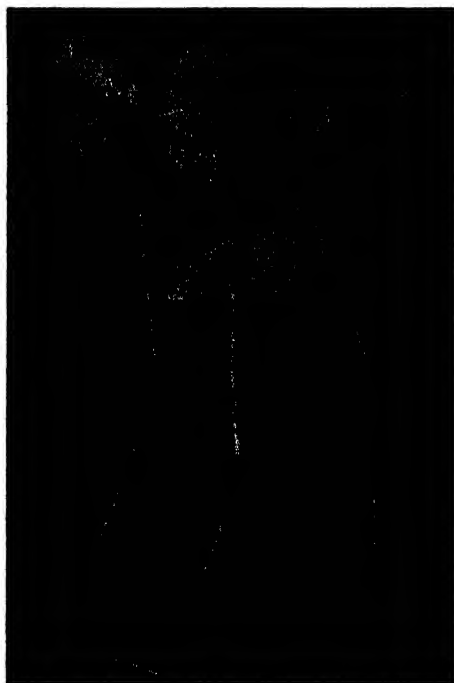


FIG. 5. Lesions on cotton seedlings produced by inoculation of seeds with *Colletotrichum indicum*.

border. Under natural conditions the infected bolls usually opened prematurely or became mummified and were shed. Resistance was increased with the maturity of the boll and decreased by mechanical injury. The bolls were also inoculated in the field by spraying with conidial suspension of the fungus, the inoculated bolls being covered with moist paper bags to maintain the humidity. No infection was thus obtained, whether the bolls were injured or not; this was probably due to the difficulty of maintaining a high humidity. Blossoms and blossom-buds, after being inoculated, were usually shed and only occasionally developed into bolls. Repeated attempts failed to produce infection of the woody stems of the mature cotton plants.

Ramakrishnan (1941) found that *Colletotrichum indicum* infects only species of *Gossypium* indigenous to India, while those introduced species, including *G. hirsutum*, *G. Davidsonii*, *G. barbadense*, *G. Armourianum*, and *G. Harknessii*, are immune to its attack. However, the two varieties of *G. hirsutum*, Trice and Delfos, used in our tests contracted the disease readily

through artificial inoculation and were also noticed as being infected occasionally in nature. It appears interesting to note that the two geographic strains of the fungus have such a difference in host range as evidenced by the con-



FIG. 6. Lesions on young leaves of cotton produced by inoculation with *Colletotrichum indicum*.

tradictory results. Of course there is the possibility that the few varieties of *G. hirsutum* tested by Ramakrishnan happened to be immune to the disease; the immunity may not be a constant specific character of the species concerned.

*Experimental host range.* Dastur (1934) was unsuccessful in infecting, even through wounds, the fruits of chili and tomato and the leaves of sugar-cane with *Colletotrichum indicum*. Ramakrishnan (1941), however, succeeded in producing, by inoculation, rot in *Capsicum* fruits and leaf spots in *Aristolochia bracteata* and *Zingiber officinale*.

In the course of the present study a number of plants were inoculated on various parts with this fungus. The seedlings of plants tested all proved immune under the experimental conditions, while fruits of pepper, tomato, and eggplant, and pods of soybean and cowpea were successfully infected. The results are summarized in Table VI.

TABLE VI

Results of Inoculations of various Plants with *Colletotrichum indicum*. A plus sign indicates a positive result

| Plant.   | Part inoculated. | Result.  |            |
|--|------------------|----------|------------|
|  |                  | Wounded. | Unwounded. |
| <i>Allium cepa</i> L.                                | Bulb             | —        | —          |
| <i>Brassica oleracea</i> L. var. <i>capitata</i> L.  | Seedling         | —        | —          |
| <i>Capsicum annuum</i> L. var. <i>groszum</i> Sendt. | Fruit            | +        | +          |
| <i>C. annuum</i> L. var. <i>longum</i> Sendt.        | Seedling         | —        | —          |
|  | Fruit            | +        | +          |
| <i>Cucumis sativus</i> L.                            | Fruit            | —        | —          |
| <i>Glycine soja</i> S. et Z.                         | Pod              | +        | +          |
| <i>Hibiscus mutabilis</i> L.                         | Young shoot      | —        | —          |
| <i>Lactuca sativa</i> L.                             | Seedling         | —        | —          |
| <i>Lycopersicum esculentum</i> Mill.                 | Seedling         | —        | —          |
|  | Fruit            | +        | —          |
| <i>Malva sylvestris</i> L.                           | Seedling         | —        | —          |
| <i>Phaseolus vulgaris</i> L.                         | Pod              | —        | —          |
| <i>Pisum sativum</i> L.                              | Pod              | —        | —          |
| <i>Raphanus sativus</i> L.                           | Pod              | —        | —          |
| <i>Solanum melongena</i> L.                          | Seedling         | —        | —          |
|  | Fruit            | +        | —          |
| <i>S. tuberosum</i> L.                               | Tuber            | —        | —          |
| <i>Vicia faba</i> L.                                 | Pod              | ?        | —          |
| <i>Vigna sinensis</i> Endl.                          | Pod              | +        | +          |

## LIFE HISTORY

In its life history *Colletotrichum indicum* does not differ essentially from other species of the genus. Since no ascigerous stage has hitherto been discovered, the fungus may overwinter in three possible ways: (1) inside the seed; (2) in the affected host tissue left in the field; and (3) in the soil. The first way appears to be, in most cases, responsible for the initial source of infection and is evidenced by the infection experiments as described above and also by the appearance of the fungus from surface-sterilized cotton seeds planted on agar plates. The possibility of the second way was proved by the following experiments. Sterilized cut-pieces of the cotton stem with the fungus growing in it were wrapped in thick paper and wire gauze and were carried through the winter under the following conditions: (1) hung on the tree in the open; (2) placed on the surface of the ground; (3) buried 5 in. deep in the soil. Isolations were made in the next spring. The fungus was recovered from the material hung on the tree and buried underground, while that left on the surface of the ground failed to show it as it was overgrown by numerous micro-organisms. Thomas (1939, 1940) and Ramakrishnan (1941) obtained evidence that the disease may be carried through the soil. Whether soil can act as a substratum for the overwintering of the fungus, however, is rather questionable. Under experimental conditions, when the soil was heavily infected with the fungus, diseased seedlings were observed in a few cases emerging from seeds planted

even 3 months after the infestation. Nevertheless, since the infection might originate from the pathogen borne inside the seeds rather than that surviving in the soil, the evidence seems to be inconclusive. Under the competition with various soil micro-organisms in nature, the chances of a facultative saprophyte like *C. indicum* surviving a long unfavourable period appear rather small.

Once the fungus has established itself through overwintered mycelium and fructifies in the host, its further dissemination will be effected by the successively produced conidia which are separated from each other and washed away from the mucilaginous matrix during rain periods. In this way, the possibility of establishing a new infection will depend upon the rapidity of germination and the resistance to drying of the conidia. As has been experimentally proved, the conidia of *Colletotrichum indicum* are rather short-lived and can only survive less than 24 hours after being dried. This, however, may be compensated by the rapidity of their germination, which usually takes place within a few hours at favourable temperatures. Thus this may bring about infections before drying can affect their vitality.

Concerning the development of the disease, this fungus responds to the environmental conditions in a similar way to *Glomerella gossypii*. In brief, fairly high temperature in combination with high humidity favour its development. In the cotton region of this province temperature in a normal year averages about 20° C. at the time of seeding and increases to about 28° C. in August; at that time, as the optimum temperature for the growth of the pathogen approaches, the disease becomes most prevalent on immature cotton bolls. Since temperature is normally sufficiently high to encourage the occurrence of the disease throughout the growing season of cotton, humidity alone remains as a limiting factor. If humidity is high at the seedling stage, the infection on the cotyledon may extend to the young stem through the petiole, and eventually bring about the death of the seedling. Nevertheless, the extension of the lesion is usually limited by the dry weather. At the boll stage the degree of rotting is also determined by the humidity. A rainy or cloudy period not only facilitates the dissemination and germination of conidia but also prevents the mucilaginous matrix from drying out, hence the conidia may survive for a longer period.

#### SUMMARY

A species of *Colletotrichum* which differs from the conidial stage of *Glomerella gossypii* in morphological characters was noticed on diseased cotyledons and bolls of cotton in Szechuan Province, West China. It was considered as identical with *C. indicum* Dast. The acervuli of the fungus measure 27–124  $\mu$  in diameter. The conidia are falcate, obtuse at the ends, and measure 16.5–27.5  $\times$  3–5  $\mu$ . The setae are produced in an early stage of growth on the stroma and are chiefly interspersed with the conidiophores.

The conidia germinate more rapidly on agar media than in water. During germination septation is usually found and 1 to 2 germ tubes are sent out, at the tips of which appressoria are produced.

The optimal temperature for the germination of conidia was about 32° C. and for the growth of the fungus on potato-dextrose agar about 28° C. The optimal pH for the germination of conidia was about 5.4, and for mycelial growth under the cultural conditions studied between 5.4 and 7.6.

The conidia are very susceptible to desiccation, surviving less than 24 hours after being dried on a glass slide, either embedded in the cirri or in water.

The production of toxic substance as the cause of blight of cotton seedlings was not confirmed in this fungus.

Artificial infection was successful on both Chinese varieties of *Gossypium arboreum* and American varieties of *G. hirsutum*. Soaking of seeds in conidial suspension of the fungus resulted in a high percentage of diseased seedlings. Lesions on the cotyledons, young stems, young leaves, and detached bolls were induced by spraying with the conidial suspension.

Besides cotton, fruits of pepper, tomato, eggplant, and pods of soybean and cowpea were successfully infected under experimental conditions, while no plant included in the tests was found susceptible at the seedling stage.

The ascigerous stage of the fungus was not discovered. The fungus overwinters chiefly inside the infected seeds and possibly in the affected host tissues left in the field.

Fairly high temperature in combination with high humidity favour the development of the disease, the latter factor being even more important under the conditions of cotton-growing regions of Szechuan. A rainy or cloudy period not only facilitates the dissemination of conidia but also prevents the mucilaginous matrix from drying out; hence the conidia may survive for a longer period.

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# On the Prothallus of *Lagenostoma ovoides* Will.

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With Plates I and II and three Figures in the Text

## INTRODUCTION

THE specimens of the Carboniferous seed *Lagenostoma ovoides* Will. to be described in this paper are of special interest in providing additional knowledge of the female gametophyte or prothallus. Examples of this seed are commonly met with in the coal-balls of the Lower Coal Measures, but nearly always they contain only very slight remains of a prothallus (if any) within the megaspore. Thus, although the general structure of the seed has been known for many years, knowledge of the prothallus has remained incomplete. It is not intended to describe in detail each of these specimens showing remains of a prothallus. The main description will be of a single well-preserved seed, referred to as specimen A. A second seed (specimen B), somewhat inferior in preservation, will be described briefly in order to confirm the main structural details seen in specimen A. In addition, a short account will be included of a number of specimens showing the prothallus in an earlier stage of development and less complete condition of preservation.

The first description of this seed was published by Williamson (1877, p. 232) at the time he established the genus. Later (1879, p. 517) another specimen was described by him. It has since received fuller treatment by Miss T. L. Pranker (1912, p. 461), and brief text-book accounts are given by Seward (1917, p. 62), Scott (1923, p. 74), and Chamberlain (1935, p. 21). Miss Benson (1908, p. 409) described a specimen suggesting that the germinating pollen grains liberated antherozoids. Koopmans (1928, pp. 1-3) has recorded the occurrence of this species in nodules from the Finefrau Nebenbank horizon of Holland and Germany and from the somewhat higher Katherina seam of Germany.

The closely related seed *Lagenostoma Lomaxi* Oliver and Scott ex Williamson was so named by Williamson in his MS. Catalogue but was not described by him. This task was undertaken by Oliver and Scott (1904, p. 193). In addition, R. C. McLean (1912, p. 305) has described an isolated transverse section showing a prothallus. Williamson also included in his genus the seed *Lagenostoma physoides* which, however, he had previously named *Physostoma elegans* (1876, p. 159). When Oliver (1909, p. 73) came to describe this seed more fully he retained Williamson's first name due to certain distinctive

characters. Again, Seward (1917, p. 64) has considered it best to exclude from the genus those species only known from impressions and casts and has placed them in the distinct genus *Lagenospermum* Nathorst. Thus the form-genus *Lagenostoma* as it now stands includes only the two species *L. ovoides* and *L. Lomaxi*.

In *L. Lomaxi* only traces of prothallial tissue were seen by Oliver and Scott (1904, p. 202) and no indication of archegonia. The section described by McLean, however, is of a well-preserved specimen in which a true parenchymatous tissue is present within the megaspore, undoubtedly representing a portion of the prothallus. It consists of a surface layer of small cells succeeded by a broad zone of radially elongated ones and a central region of rounded cells; no archegonia, however, are present. Seward (1917, p. 60) and Scott (1923, p. 69) state that archegonia are not known, though Chamberlain (1935, p. 21) says that '... some of the seeds show traces of the female gametophyte and even enough of the archegonia to show that they are of the elongated type'. It is not known on what evidence this statement is founded.

In *L. ovoides* less is known of the prothallus than in *L. Lomaxi*. None of Williamson's specimens showed its presence, while, although Miss Pranker (1912, p. 479) described four sections interpreted as showing early stages in the development of the prothallus, none of them possessed clearly defined cells, though they suggested that development was centripetal and comparable with that of modern cycads. These specimens are closely similar to the five described briefly in the latter part of this paper.

## DESCRIPTION OF SPECIMEN A

### *The Sections*

This specimen occurred in a small calcareous nodule obtained on old tips at Cloughfoot, Dulesgate, Todmorden, the locality from which the section of *L. Lomaxi* described by McLean also came. The seed was discovered only after grinding into the chalazal end. Twenty-nine serial sections were made by Walton's peel method; these represent about two-thirds of the entire specimen. The sections are obliquely transverse, at about  $29^\circ$  to the horizontal. Though this obliquity is not very apparent in the lower sections, it becomes more marked in the apical region. On Pl. I, Figs. 1–13, photographs of the principal sections are shown. They are all similarly orientated relative to one another, the highest point of each section lying towards the top left-hand corner of the plate. After considering the sections the method of determining the angle of obliquity and of making the reconstruction shown on Text-fig. 2 will be described.

#### (a) *The region of the prothallus below the archegonia.*

This region is represented in sections 1 to 15, and of these, sections 1 and 12 are shown on Pl. I, Figs. 1 and 2. In each the prothallus is surrounded by a thin, black, structureless membrane from which it has retracted round a

part of its circumference. At points the membrane separates into two layers, a fact which suggests that it is probably of a double nature and represents the megaspore wall fused with part of the nucellus (Pl. I, Fig. 1, M). Another thin black layer still adhering to the inner surface of the integument no doubt represents the remainder of the nucellus.

It is evident from the appearance of the prothallus in successive sections that it underwent maximum contraction in the basal region. In section 1 (Pl. I, Fig. 1) the prothallus is in contact with only about one-quarter of the megaspore wall, leaving a crescentic cavity on one side. Its free surface is interrupted by a split-like incision interpreted as an artifact. The cells are larger and more clearly defined towards the side in contact with the megaspore wall. At the free surface the cells lack their outer walls, but where the prothallus abuts on the megaspore membrane true outer walls are present. There is evidence in some of the sections that the absence of outer walls from peripheral cells is due to tearing away from the megaspore membrane, to which some of these walls can be seen still adhering. The same condition was noticed by McLean (1912, p. 308) in *L. Lomaxi* and suggests that the prothallus originally filled the megaspore but contracted, especially in the basal region, either before or during preservation.

Towards the side of the prothallus adpressed to the megaspore wall there is in the lower sections a slight differentiation of the tissue into two zones. This becomes more pronounced in the higher sections where it affects the whole sectional area of the prothallus (Pl. I, Fig. 2), but apparently it disappears towards the apex. Where the differentiation is greatest two regions can be distinguished. The first is an outer zone of radially elongated cells, the outermost of which are often the smallest, though it cannot be said that they constitute a clearly organized peripheral layer since occasionally the elongated cells also abut on the surface. In section 12 (Pl. I, Fig. 2) the maximum size of these radially elongated cells is  $0.14 \times 0.04$  mm. The zone itself is at the most 0.38 mm. wide and consists of radial rows of from three to five cells. Secondly, within the radial zone is the central region of rounded or polyhedral cells measuring from 0.05 to 0.07 mm. in diam. These cells constitute the bulk of the prothallial tissue.

The slit-like incision present in section 1 persists in the series as far as section 10, after which it terminates as two small central cavities seen in section 12 (Pl. I, Fig. 2). In the next three sections contraction has been negligible, the prothallus appearing as a solid mass of tissue filling the cross-sectional area of the megaspore.

#### (b) *The terminal region of the prothallus.*

This region of the prothallus extends through sections 16 to 25. It is characterized by the presence of the archegonia and the apparent absence of an outer zone of radially elongated cells. However, if such cells were present in this region they might tend to be arranged more or less longitudinally. If so the sections would cut them transversely or obliquely. This appearance is



suggested in the lower of these sections but there is no evidence of it in the higher ones, where the cells are smaller and less clearly defined, as at the base of the prothallus.

Up to the level of section 19 the prothallus fills the megaspore, but in sections 20 and 21 (Pl. I, Figs. 5 and 6) a cavity appears on one side between the prothallus and megaspore wall. From the preceding sections it is evident that this cavity was immediately above the first archegonium to appear in the series. Another cavity between the megaspore wall and plinth also first appears in section 20 (Pl. I, Fig. 5). The presence of these cavities on one side only shows clearly that the sections are oblique. Sections 22 to 25 (Pl. I, Figs. 7 to 10) show an increase in the extent of the cavity between the megaspore wall and plinth, and simultaneously the central region of the prothallus becomes delimited from the rest as a terminal prolongation projecting into the lower part of the cavity of the lagenostome. In sections 22 and 23 (Pl. I, Figs. 7 and 8) this projection measures 0.45 and 0.36 mm. in diam., respectively, and still abuts on the rest of the prothallus on one side, due to the oblique nature of the sections. In section 24 (Pl. I, Fig. 9) it occurs as an almost separate circular patch of tissue 0.28 mm. in diam. and, as the reconstruction in Text-fig. 2 shows, this would be the only part of the prothallus to appear in a truly transverse section at this level. Section 25 (Pl. I, Fig. 10) confirms this, since part of the upper extremity of the apical prolongation occurs separate from the rest of the prothallus as a small isolated patch of tissue. The interpretation placed upon this structure is that it corresponds to the 'tent-pole' occurring in several other palaeozoic seeds and in Ginkgo among living gymnosperms.

The megaspore wall is very difficult to follow in these later sections. It is clearly shown in section 20 (Pl. I, Fig. 5, M) where it is still intact, but in sections 21 and 22 (Pl. I, Figs. 6 and 7) it is more irregular and indefinite. Above this level it may have been broken; thus in section 24 (Pl. I, Fig. 9) there is no sign of it around the apical prolongation of the prothallus.

The tissue of the lagenostome known as the central column is only present in the upper region. It appears first in section 26 (cf. section 27, Pl. I, Fig. 11). Like other structures it first appears to one side. At this level it is hollow, somewhat angular, and incomplete in outline. Except at one point, it lies free from the wall of the lagenostome. The tissue in its lower part appears to have disintegrated considerably. In the higher sections, however, it is solid and composed of dense, thick-walled cells. In this region the angular wall of the lagenostome fits very closely round the central column and is surrounded in turn by the chambered canopy of the integument consisting of eight lobes. This is the appearance as seen in the last section of the series (cf. Pl. I, Fig. 12).

### (c) *The archegonia.*

The three structures here described as archegonia do not appear simultaneously in the series, due to the obliquity of the sections. The reconstruc-

tion on Text-fig. 2 shows that they occur actually at approximately the same level in the seed. The first archegonium to appear in the series lies nearer to the side on which the plinth and canopy first appear than does the second, and the latter bears a similar relationship to the third. On the sections they are separated from one another laterally by distances up to about three times their average maximum diameter. Each consists of an egg-cell estimated to be about 0.3 mm. in length and surrounded by a definite layer of cells, the archegonial jacket. The preservation does not permit of the distinction of neck-cells or ventral canal cells. The reconstruction (Text-fig. 2) shows that they opened on the shoulder of the prothallus around the 'tent-pole'. The positions assigned to them in the reconstruction represent the condition in the solid prothallus since only one of them (No. 3) occurred in the plane of the median longitudinal section reconstructed. The first actually lies anterior to the plane of the reconstruction and the second is posterior to it. Each archegonium extends through three sections. The maximum diameter of the first and second egg-cells is 0.19 mm.; this is attained in the second section through each (Pl. I, Figs. 3 and 5). The third egg-cell attains a maximum diameter of 0.29 mm. in the highest section through it (Pl. I, Fig. 8).

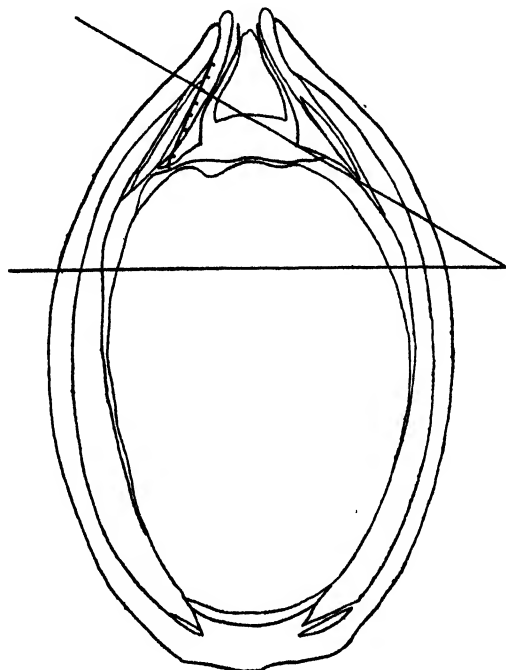
The appearance of the jacket cells is shown most clearly on Pl. I, Fig. 13. This is a reproduction, at higher magnification, of the first egg-cell shown on Pl. I, Fig. 3. The cells of the jacket are rectangular or square in section. They form a layer 0.024 mm. wide and are smaller than the surrounding prothallial cells which measure 0.03 to 0.06 mm. in diameter. They also appear to have thicker cell-walls, but there is no indication of the pitting present on the inner walls of this layer in such recent gymnosperms as *Cycas*.

The preservation does not permit of the distinction of a neck in any of the archegonia. Between the first egg-cell and the periphery of the prothallus in section 18 (Pl. I, Fig. 4) the tissue is slightly broken down. Likewise, to the outside of the second egg-cell in section 20 (Pl. I, Fig. 5) there is a slight indentation covered in by the megaspore wall. However, since these have more of the appearance of artifacts and are laterally placed relative to the egg-cells, they probably had no connexion with the opening of the archegonia to the surface.

### *The Reconstruction*

Text-fig. 2 represents a reconstruction and partial restoration of a median longitudinal section of specimen A. Before it was possible to make this reconstruction the angle of obliquity of the sections had to be determined. This could only be done approximately, the method adopted being illustrated in Text-fig. 1. A line-diagram of a typical seed cut longitudinally was first drawn. For this a section of specimen E shown on Pl. I, Fig. 23, and described later, was used. This section appears to be truly median and its preservation is remarkably good. It is known that in specimen A nine sections were made between the level at which the plinth first appears (section 20, Pl. I, Fig. 5) and that in which the upper extremity of the cavity in the canopy occurs on the

same side of the sections (section 28, Pl. I, Fig. 12). On the drawing of specimen E (Text-fig. 1) these two levels were joined by a line which was then divided into eighths according to the number of sections made through this

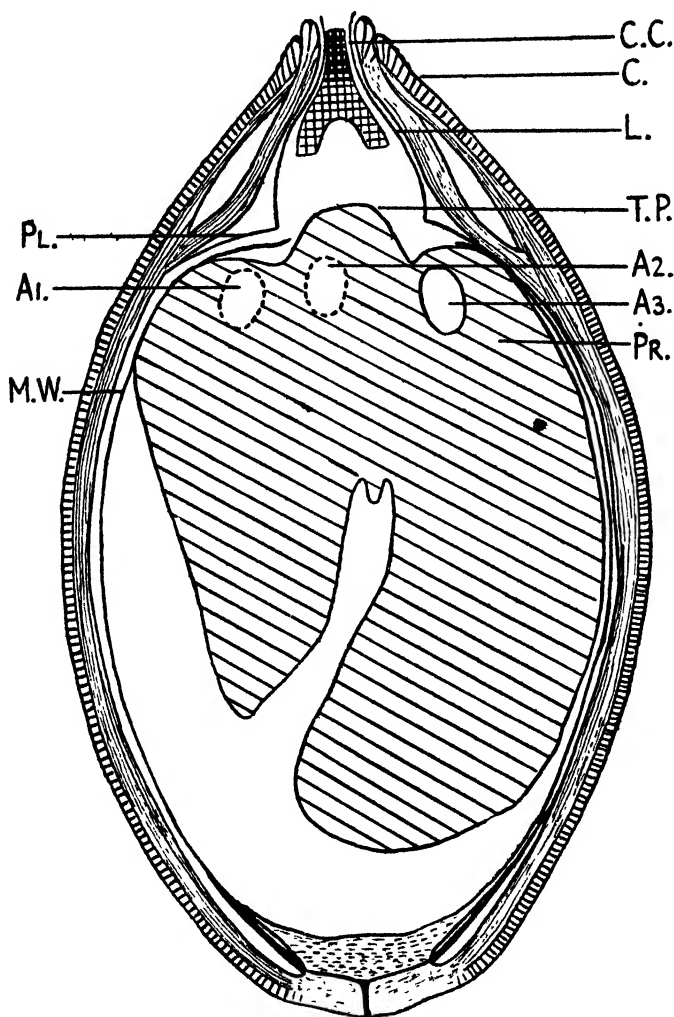


TEXT-FIG. 1. Line-diagram of a median longitudinal section through a seed of *L. ovoides* (specimen E) to illustrate the method of determining the angle of obliquity of the sections through specimen A. ( $\times 20$ .) For further explanation see text.

region in specimen A. It is also known that the base of the lagenostome (in specimen A) first appears in a complete condition on the opposite side of the seed in section 26. Hence on the drawing (Text-fig. 1) a line was constructed from the base of the lagenostome on the right-hand side to the level determined for section 26 on the left-hand side. This gives the plane of one section which proves to be  $29^\circ$  to the horizontal. To ascertain the position of the remaining sections they were taken to be parallel to section 26 and equidistant from each other. Since it was not known how far apart consecutive sections were, the distance was arbitrarily taken to be the same as that determined for the nine sections through the lagenostome region based on specimen E. This gave a value of  $0.1$  mm., which is probably slightly too large, since specimen E was slightly larger than specimen A. After fixing the approximate planes of the sections the reconstruction could be built up. Camera-lucida drawings of all the sections were first made. A line was then drawn on each representing the maximum diameter. The positions of the various structures on this diameter were then transposed to the level of each section previously deter-

mined. Thus the positions of the structures as they would appear on a median longitudinal section were ascertained.

The maximum width of the seed was taken as the minimum diameter of the



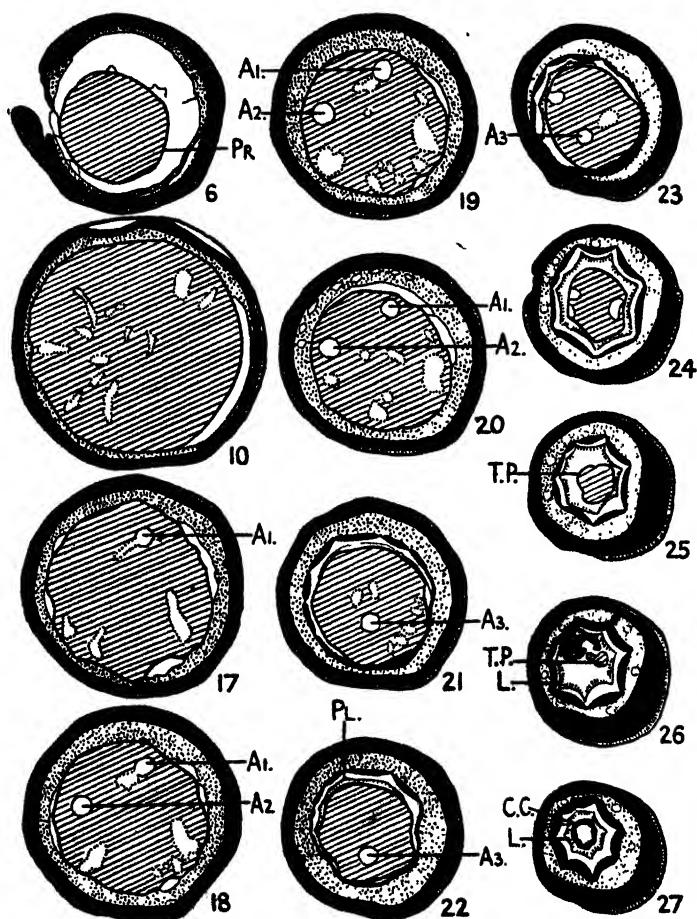
TEXT-FIG. 2. Reconstruction and partial restoration of a median longitudinal section through a specimen of *L. ovoides* containing a mature prothallus. (Specimen A.) ( $\times 30$ .) The shading of the prothallus indicates the angle of the sections from which the reconstruction was made. A1, A2, and A3 = 1st, 2nd, and 3rd archegonia; C. = canopy; C.C. = central column; L. = lagenostome; M.W. = megaspore wall; PL. = plinth; PR. = prothallus; T.P. = 'tent-pole'.

largest section in the series and measured 2.5 mm. as compared with 2.7 mm. for specimen E. Since the latter measures 4.4 mm. in length, the length of specimen A was probably about 4 mm. Only about two-thirds of the seed could be reconstructed in this way since the chalazal region had been lost by

grinding before the seed was discovered. This region was therefore restored from its appearance in sections of other specimens.

#### DESCRIPTION OF SPECIMEN B

This specimen was discovered in a small nodule from Bacup, Lancashire. The nodule was cut more or less at random, but the section happened to pass



TEXT-FIG. 3. Camera-lucida diagrams of the principal sections in a series through a seed of *L. ovoides* possessing a mature prothallus. (Specimen B.) ( $\times 13$ .) A1, A2, and A3 = archeogonia; C.C. = central column; L. = lagenostome; PR. = prothallus; PL. = plinth; T.P. = 'tent-pole'. The number attached to each diagram refers to that of the section of which it is a drawing.

approximately through the middle of the seed in a transverse direction. Unfortunately the preservation is somewhat inferior compared with that of specimen A, but it is adequate to confirm the main features seen in that specimen.

Camera-lucida diagrams of the principal sections are shown on Text-fig. 3 and photographs of five of these sections are shown on Pl. I, Figs. 14 to 18. It is not necessary to describe the seed in detail, but the main features, all of which agree with specimen A, can be summarized thus. (1) The prothallus had contracted in the basal region (Text-fig. 3, section 6 and Pl. I, Fig. 15), but in the higher sections it more or less fills the megaspore. (2) In the widest region of the seed the prothallial tissue shows a differentiation into an outer zone of radially-elongated cells and an inner mass of polygonal cells. There is, however, slight indication of a more distinct peripheral layer of small cells than in specimen A (Pl. I, Fig. 14). (3) Three archegonia are present, each showing a distinct archegonial jacket but it does not appear how they opened to the surface (Pl. I, Figs. 16 and 17). (4) The first archegonium is present on sections 17 to 20, the second on sections 18 to 20, and the third on sections 21 to 23 (see Text-fig. 3). Although there are many lacunae also in the prothallus, the archegonia can readily be distinguished from these by the surrounding layer of small jacket cells. (5) There is evidence of a 'tent-pole' projecting into the base of the lagenostome (Text-fig. 3, sections 25 and 26, and Pl. I, Fig. 18). (6) The tissue of the central column has disintegrated in the lower region of the lagenostome and only the upper indurated portion has persisted. (7) The maximum diameter of the seed was 2.5 mm. and the angle of the sections was estimated at about  $10^{\circ}$  from the horizontal.

#### SPECIMENS C AND D

The two specimens referred to here as specimens C and D were found in coal-balls from old tips at Shore, Littleborough, Lancashire.

Specimen C was exposed at the surface of a small nodule and was ground in such a way as to give longitudinal sections, one of which is shown on Pl. I, Fig. 19. It contains a megaspore almost filled with diffuse carbonaceous matter presenting a peculiar spotted appearance. This is presumably the remains of a prothallus. However, as there are no true cell walls the problem arises as to whether the appearance is due simply to inferior preservation of what was originally a truly cellular prothallus or whether it represents some pre-cellular stage of development. The conclusion arrived at will be stated later, after consideration of the other specimens.

The apical region of the prothallus is reproduced at a higher magnification on Pl. I, Fig. 20, and is seen to protrude slightly into the base of the lagenostome. It is by no means certain, however, that this bulge is representative of a 'tent-pole' since the adjacent sections of the series show no signs of it.

Below the apical bulge and to one side is an ovoid body of dense carbonaceous matter surrounded by a clear space. Its position suggests an archegonium, but the absence of other similar bodies leaves the suggestion without satisfactory confirmation.

A remaining point of interest in this seed may be noted. The cavity of the lagenostome is filled in its lower part with a loose web of hyphae presumably

of a saprophytic fungus, a small portion of which is shown at a higher magnification on Pl. I, Fig. 21. The central column has apparently undergone disintegration in this lower region, and where present in the upper region its appearance also suggests partial disintegration.

Specimen D (Pl. II, Fig. 22) is a very oblique section through a seed essentially similar to C. The prothallus presents the same spotted appearance, and again there is a small body of denser carbonaceous matter in a position suggestive of an archegonium. The central column shows disintegration—hyphae of a fungus being present in both the lagenostome and canopy. There is no evidence of a 'tent-pole'.

#### SPECIMENS E, F, AND G

These specimens were found along with others in nodules obtained at Nabb Colliery near Bacup, Lancashire.

Specimen E is shown on Pl. II, Fig. 23. Twenty-three longitudinal sections were made through the seed, the section photographed being apparently perfectly median. The appearance of the prothallial remains is again similar to specimen C, but the carbonaceous matter is more sparse. There are no signs of archegonia and no satisfactory indication of a 'tent-pole'. The central column of the lagenostome is, however, well preserved and no fungal hyphae can be seen. The fact that the central column is well preserved suggests that the conditions must have been suitable also for equally good preservation of mature prothallial tissue. The absence of any such tissue possessing cell walls seems to suggest therefore that the prothallus had not reached a stage comparable with specimens A and B.

Specimen F is shown on Pl. II, Figs. 25–7. Sixteen very oblique sections were made through this seed. It shows more clearly than the others a radial arrangement of the spots of carbonaceous matter lying towards the outside (Pl. II, Fig. 27). Within this zone the spots show no radial arrangement, whilst the central area is almost devoid of prothallial remains. In the apical region there are three ovoid bodies interpreted as archegonia; two are shown faintly on Pl. II, Fig. 25 and the third on Pl. II, Fig. 26. Their position conforms to that seen in specimens A and B. Around one of these archegonia are definite indications of cells with walls. There seems to be no doubt therefore that the prothallus was cellular, but the fact that only few cell walls were preserved suggests that they were more delicate than those in specimens A and B. The appearance of some of the radially arranged spots of carbonaceous matter is also strongly suggestive of cell protoplasts with dense contents, the intervening cell walls having disappeared. The apex of the prothallus shows no indication of a 'tent-pole' but fits closely under the lagenostome. In the latter the tissue of the central column is well preserved and has not undergone much disintegration.

Specimen G is shown on Pl. II, Fig. 24. Five transverse sections only were obtained through this seed. They show a contracted prothallus in which

obscure rounded cells with walls can be distinguished towards the outside. The central region is more or less filled with diffuse carbonaceous matter presenting the typical spotted appearance seen in the other sections.

# CONCLUSION

The general conclusion drawn from the brief survey of the additional specimens is that they represent a fairly early stage of a truly cellular prothallus in which most of the cell walls were probably too delicate to be preserved. Dense cell contents appear to have been present in many of the cells. Towards the periphery the cells were apparently elongated radially and arranged in radial rows. Archegonia were sometimes present, but there was no definite 'tent-pole'. It would appear, therefore, that the prothallus only possessed a 'tent-pole' when mature and that by the time it had been differentiated the lower part of the central column had disintegrated. This may be compared with *Conostoma oblongum* Will. in which there was also a 'tent-pole' and probably a natural dissolution of tissue in the lagenostome (Oliver and Salisbury, 1911, p. 21). The presence of a 'tent-pole' is also shown by several other palaeozoic seeds, notably *Gnetopsis elliptica* Renault, *Stephanospermum akenioides* Br., *Trigonocarpus pusillus* Br., and a number of the *Cardiocarpales*.

Among recent gymnosperms *Ginkgo biloba* possesses a female gametophyte with two, occasionally three, archegonia opening into a circular crevice around the base of the 'tent-pole' (Hirasé, 1898, p. 113). Moreover, the cells at the apex of the nucellus become brown in colour and persist as a solid protuberance on top of the nucellus, while the lower cells round the pollen chamber break down, forming a cavity which surrounds the 'tent-pole'. These characters would seem to find a parallel in *L. ovoides*. A more general resemblance also exists between *L. ovoides* and other recent gymnospermous seeds in the radial arrangement of the outer prothallial cells; this is probably indicative of a centripetal mode of development.

# SUMMARY

The mature prothallus of *L. ovoides* Will. is described from a well-preserved specimen, supplemented by a brief account of a second specimen of somewhat inferior preservation.

There is a partial differentiation of tissue into outer radially elongated cells and inner polyhedral or rounded cells.

Three archegonial egg-cells are present. They are ovoid in shape and possess a distinct layer of jacket cells but no neck cells can be distinguished.

A 'tent-pole' prolongation of the prothallus is present. It projects into the base of the lagenostome, the lower part of the central column having apparently disintegrated.

Five additional specimens are described briefly and interpreted as showing an earlier stage of a truly cellular prothallus incompletely preserved, due probably to the delicate nature of the cell-walls.



In this earlier condition of the prothallus archegonia may be present but the 'tent-pole' is either absent or only very slightly developed.

Finally the writer wishes to acknowledge his indebtedness to Prof. W. H. Lang and Prof. C. W. Wardlaw for generous assistance given in various ways connected with this work, and to Mr. E. Ashby for his skill in the taking of the photographs.

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#### EXPLANATION OF PLATES I AND II

Illustrating Mr. Long's article 'On the Prothallus of *Lagenostoma ovoides* Will.'

##### PLATE I

Fig. 1. Specimen A. Obliquely transverse section through basal region of seed showing contracted prothallus. ( $\times 20$ .) (Section 1.) M = megaspore membrane.

Fig. 2. Specimen A. Section through middle region of seed showing prothallus. ( $\times 20$ .) (Section 12.)

Fig. 3. Specimen A. Section through terminal region of prothallus showing 1st archegonium. ( $\times 20$ .) (Section 17.) A1 = 1st archegonium.

Fig. 4. Specimen A. Section through terminal region of prothallus and 1st archegonium. ( $\times 20$ .) (Section 18.)

Fig. 5. Specimen A. Section through terminal region of prothallus showing 2nd archegonium. ( $\times 20$ .) (Section 20.) A2 = 2nd archegonium, M = megaspore membrane.

Fig. 6. Specimen A. Section through terminal region of prothallus showing 2nd and 3rd archegonia. ( $\times 20$ .) (Section 21.) A2 = 2nd archegonium, A3 = 3rd archegonium, PL. = plinth.

Fig. 7. Specimen A. Section through apex of prothallus. ( $\times 20$ .) (Section 22.) T.P. = 'tent-pole', A3 = 3rd archegonium.

Fig. 8. Specimen A. Section through apex of prothallus. ( $\times 20$ .) (Section 23.) T.P. = 'tent-pole', A<sub>3</sub> = 3rd archegonium.

Fig. 9. Specimen A. Section through plinth region of seed. ( $\times 20$ .) (Section 24.) T.P. = 'tent-pole'.

Fig. 10. Specimen A. Section through plinth region of seed. ( $\times 20$ .) (Section 25.) T.P.A. = 'tent-pole' apex.

Fig. 11. Specimen A. Section through lagenostome region of seed. ( $\times 20$ .) (Section 27.) C.C. = central column, L. = lagenostome.

Fig. 12. Specimen A. Section through apical region of lagenostome. ( $\times 20$ .) (Section 28.) C.C. = central column.

Fig. 13. Specimen A. Section through an archegonial egg-cell. ( $\times 100$ .) (Section 17.) J.C. = jacket cells.

Fig. 14. Specimen B. Transverse section through middle region of seed showing prothallus. ( $\times 20$ .) (Section 9.)

Fig. 15. Specimen B. Section through basal region of seed showing contracted prothallus. ( $\times 20$ .) (Section 6.)

Fig. 16. Specimen B. Section through terminal region of prothallus. ( $\times 20$ .) (Section 19.) A<sub>1</sub> = 1st archegonium, A<sub>2</sub> = 2nd archegonium.

Fig. 17. Specimen B. Section through terminal region of prothallus. ( $\times 20$ .) (Section 21.) A<sub>3</sub> = 3rd archegonium.

Fig. 18. Specimen B. Section through lagenostome region of seed. ( $\times 20$ .) (Section 25.) T.P. = 'tent-pole'.

Fig. 19. Specimen C. Median longitudinal section of seed showing an immature prothallus. ( $\times 20$ .)

Fig. 20. Specimen C. Apex of prothallus and part of lagenostome. ( $\times 100$ .) A = possible archegonium.

Fig. 21. Specimen C. Fungal hyphae from cavity of lagenostome. ( $\times 400$ .)

## PLATE II

Fig. 22. Specimen D. Obliquely longitudinal section mainly through upper half of seed. ( $\times 20$ .) (Section 5.)

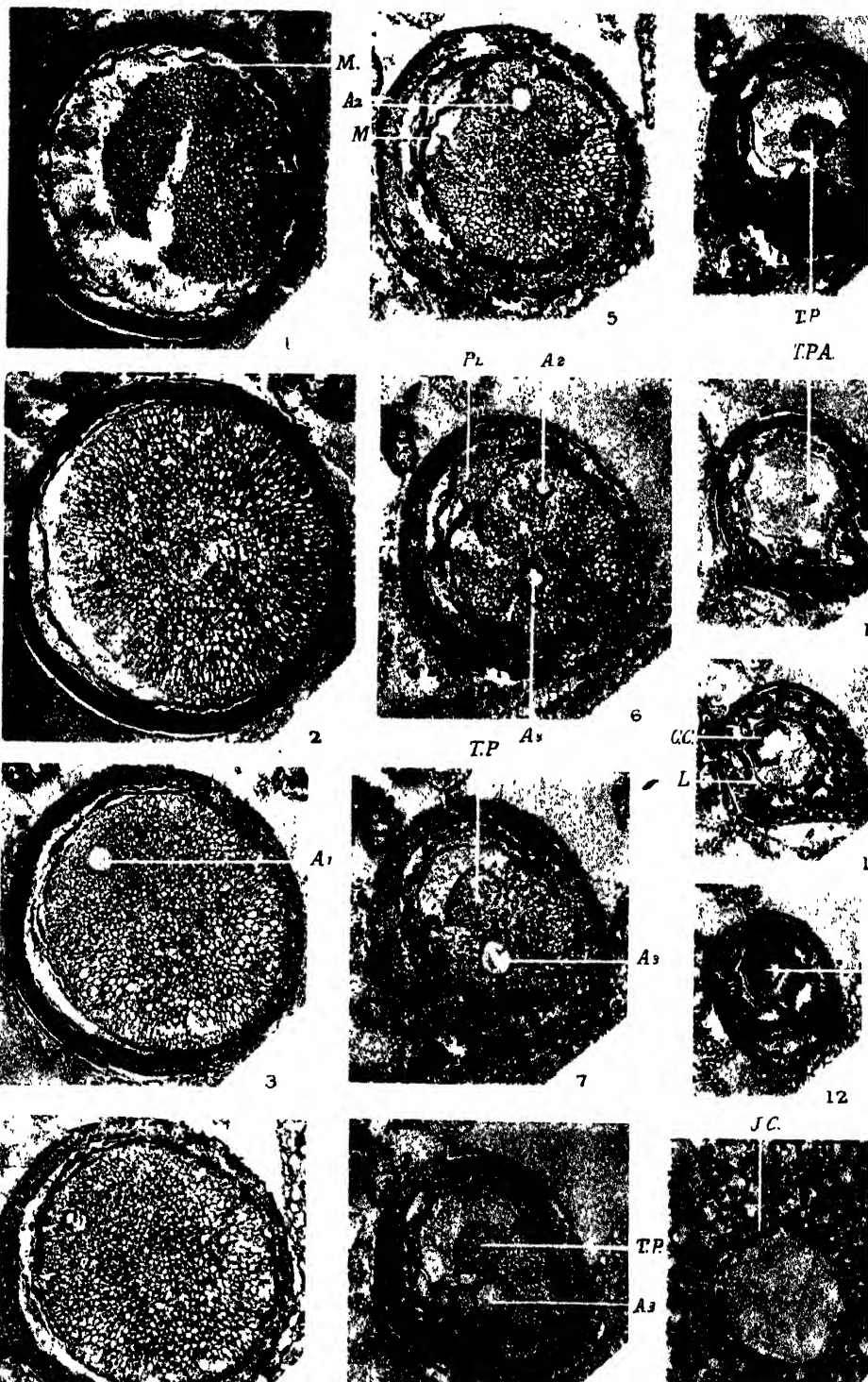
Fig. 23. Specimen E. Median longitudinal section through seed showing slight prothallial remains. ( $\times 20$ .) (Section 9.) C.C. = central column.

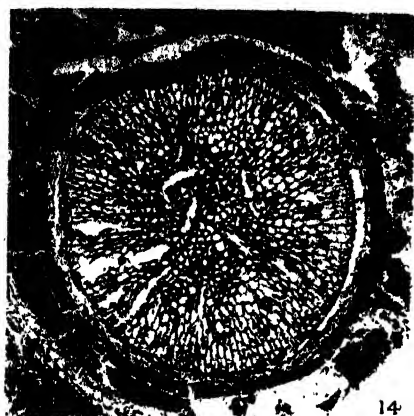
Fig. 24. Specimen G. Transverse section of seed with contracted prothallus. ( $\times 20$ .)

Fig. 25. Specimen F. Obliquely longitudinal section through lagenostome region of seed. ( $\times 20$ .) (Section 10.) C.C. = central column, A<sub>1</sub> = 1st archegonium, A<sub>2</sub> = 2nd archegonium.

Fig. 26. Specimen F. Obliquely longitudinal section of seed below the lagenostome. ( $\times 20$ .) (Section 7.) A<sub>3</sub> = 3rd archegonium.

Fig. 27. Specimen F. Obliquely longitudinal section of seed in middle region. ( $\times 20$ .) (Section 5.)

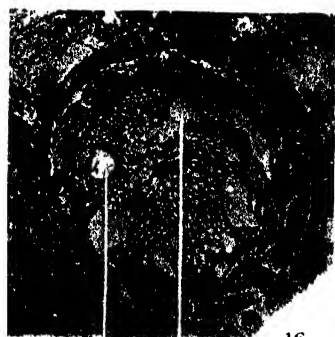




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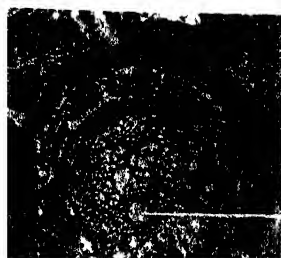


15



16

$A_2$   $A_1$



$A_3$



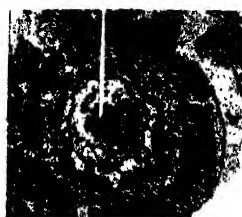
19



20

$A$

$TP$







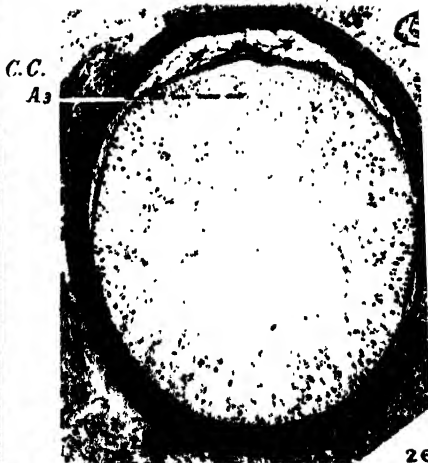
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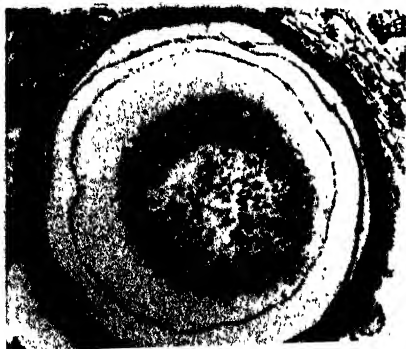
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23



26





# Polarized Segregation in an Ascomycete

BY

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With three Figures in the Text

MENDEL'S second law stated that different pairs of factors segregate independently of one another. This independence was later found not to be a general rule, although it held for many factors. The exceptions are those dependent on the phenomenon of linkage, the basis of which lies in the location of the linked genes in the same pair of chromosomes. The basis of Mendel's second law lies in the location of the independently segregating genes in separate pairs of chromosomes. It requires that the bivalents should orient themselves independently on the spindle with the two chromosomes of a bivalent directed indifferently with respect to the two spindle poles. This indifferent orientation has been assumed as an axiom, and little reason to doubt its general truth has been forthcoming.

Exceptions could theoretically be detected in various ways. Deviations from the standard ratios could be due to the effects of non-random orientation of a bivalent, but it would usually be difficult to exclude other disturbances of the ratio arising from differential viability of gametes and zygotes. The latter disturbances would include the special cases of the Renner effect of megaspore competition and replacement in the formation of the embryo-sac and of differential pollen-tube growth rates in plants.

The difficulties surrounding the detection of preferential segregation largely disappear in those cases where all the products of each meiosis can be recovered together. The best possible information would be that derived from a linear tetrad with an identifiable base and apex, as in certain Ascomycetes. Most of the published genetical studies on Ascomycetes, however, do not set out the data in such a way that non-random orientation could be detected, though doubtless the relevant information exists in the research note-books. Fortunately one large mass of data on *Bombardia lunata* has been published in detail (Zickler, 1934). It clearly demonstrates what appears to be non-random orientation leading to preferential segregation of certain genes. This feature of the data was overlooked by Zickler and apparently has not been noticed by subsequent writers on fungal genetics.<sup>1</sup>

<sup>1</sup> Ryan (1943, Bull. Torrey Bot. Club, lxx. 605-11) discusses Zickler's assertion that his data require second-division as well as first-division segregation of homologous centromeres. It is shown that no evidence exists for such a behaviour.

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## DESCRIPTION OF THE OBSERVATIONS

*Bombardia lunata* is a coprophilous Pyrenomycete belonging to the Sordariaceae. It was found by Zickler to be heterothallic, so that controlled crosses between genetically different types were possible. Besides the sex genes a number of mutants affecting such characters as the growth form and colour of the mycelium was found. Two of these, namely rubiginosa and lactea, also alter the ascospore colour.

The colour of the mycelium in the normal wild type is described as being a uniform greyish-green. Zickler refers to the normal form as viridis when he is speaking of its colour and as lanata when speaking of its mycelial growth type. It would seem more in accord with ordinary genetical practice to describe it simply as the normal or wild type and designate it by the symbol +; this practice will be followed in the present paper. The mutant rubiginosa (*r*) has a red-brown mycelium which is very vigorous. It arose as a spontaneous sector in a culture of the normal form. The mutant lactea (*l*) has an almost completely colourless mycelium with a very weak, yellowish ground colour. Lactea has arisen several times from rubiginosa as spontaneous sectors, and has also been obtained from a normal strain by X-radiation.

In the normal fungus the ascospore colour is dark, but in both rubiginosa and lactea it is light. The ascospores of the normal strain are described as being dark greenish-brown, while those of rubiginosa are bright, reddish-brown, and transparent. The ascospores of lactea are not specifically described, but photomicrographs indicate that they are transparent like those of rubiginosa. The colours apparently depend upon cytoplasmic pigments.

When either a rubiginosa or a lactea strain is crossed with an appropriate normal strain, + or — according to the 'sex' of the mutant strain, the asci produced in the resulting perithecia show segregation for ascospore colour. Thus, of the eight ascospores in any ascus, four appear dark like those in a normal strain and four light like those in the mutant strain. The arrangements of the two kinds of ascospores in the asci will be described and analysed shortly, but it is important first of all to note that the colour of the ascospore is determined by its own genotype. There appears to be no maternal influence exercised by the cytoplasm of the hybrid mycelium on which the asci are borne. The case is entirely analogous in this respect with the determination of the carbohydrate reserve in the pollen-grains produced by a maize plant segregating for waxy (*wx*) and non-waxy (*Wx*). In that case, half the pollen-grains contain erythrodextrin (*wx*), staining red with iodine (Demerec, 1924; Brink and McGillivray, 1924). A similar case occurs in rice (Parnell, 1921). Hence, in maize and rice and in the fungus *Bombardia lunata* we are enabled to observe a gametic segregation ratio directly. The fungus, however, shows an added advantage, since all the products of each meiosis are kept together within each ascus. Moreover, the fact that the ascospore nuclei are produced in a definite linear order, which they preserve until maturity, allows of more far-reaching deductions than the verification of Mendel's first law.

When the asci produced by a normal  $\times$  lactea or a normal  $\times$  rubiginosa cross are examined it is found that, with very rare exceptions, they fall into one or other of six classes, numbered 1 to 6 by Zickler and illustrated diagrammatically in Fig. 1, which is adapted from Zickler's Fig. 7. The interpretation of these arrangements of the ascospores in the asci of Ascomycetes is now well understood. It was first discussed by Dodge (1927) and most recently in detail by Whitehouse (1942). For convenience of reference the eight ascospores may be assigned the letters *a* to *h* in order from the top to the bottom of the ascus.

The ascus arises from the binucleate penultimate cell of a crozier-shaped hypha. The evidence shows that each of the two nuclei is haploid and of different genetic constitution in heterothallic Ascomycetes. The two nuclei fuse, and the resulting diploid nucleus then undergoes the first and second divisions of meiosis to give a linear tetrad of four haploid nuclei. The manner of production and the resulting arrangement of these four nuclei is such that the plane of the first-division segregation separates the upper two nuclei from the lower two nuclei.

Sooner or later each of the four nuclei divides mitotically, a linear row of eight nuclei being formed. The third-division spindle is obliquely inclined to the ascus axis or parallel to it. In either case there is no slipping of non-sister nuclei past one another at the third division. A wall is then formed around each nucleus, enclosing a mass of cytoplasm with the nucleus. An ascospore is matured from each cell so formed. As a result the planes of the first and second meiotic segregations are marked out. The plane of the first division lies between spores *d* and *e*, separating spores *a* to *d* from spores *e* to *h*. The planes of the second divisions separate spores *a* and *b* from spores *c* and *d*, and spores *e* and *f* from spores *g* and *h*. There is no genetic evidence that the two spores of the respective pairs *ab*, *cd*, *ef*, and *gh* ever differ genetically from one another, as would be expected if the third division in the ascus were reductional (cf. Dodge, 1927).

Second-division segregation occurs as the result of a cross-over between the locus of the gene-pair concerned and the centromeres of the chromosome pair bearing the genes. The proportion of second-division segregation measures directly the minimum frequency of chiasma formation between the centromeres and the locus of the gene-pair considered. The actual frequency of chiasmata in the interval may be considerably higher, especially if the interval is at all long, since the occurrence of two reciprocal chiasmata within the interval would result in the appearance of a first-division segregation. The theoretical limiting value of 66.7 per cent. second-division segregation is closely approached in the case of rubiginosa (64 per cent. found), but no very precise deductions can be drawn therefrom in the absence of information about chiasma and chromatid interference. We can say, however, that the locus of rubiginosa is at some considerable distance from the centromere.

Zickler's data (Table I on p. 591 of his paper) for the segregational arrangements of dark and light ascospores in the cross normal  $\times$  rubiginosa are reproduced in Table I. The scoring was based on eight separate samples which

are given individually in the table together with their various totals. It should be noted that the totals of the six segregation types as given by Zickler under the diagrams in his Fig. 7 are in error, in a few cases, as compared with his Table I. The latter is internally consistent, whereas the numbers in his Fig. 7

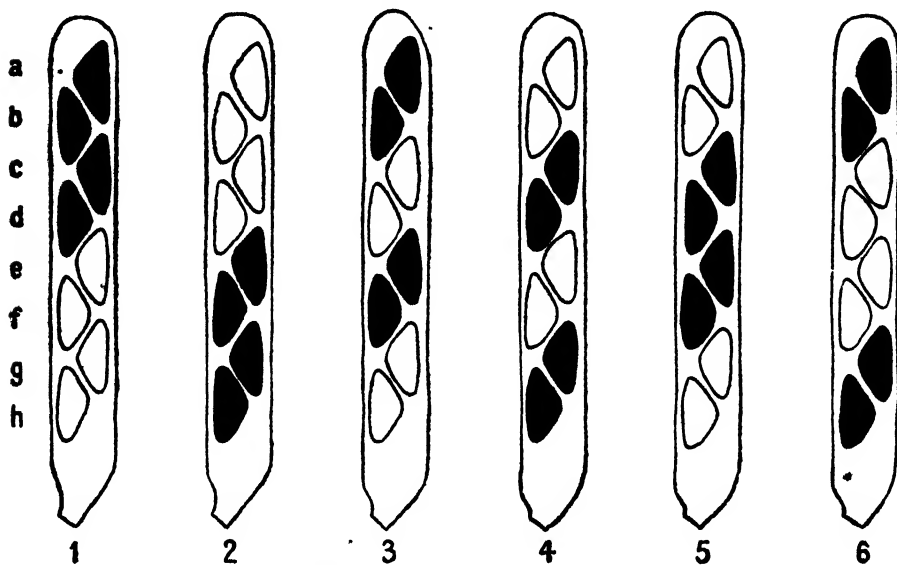


FIG. 1. Diagram to show the six ascospore arrangements. The colourless spores carry the rubiginosa, or lactea, gene, and the black spores its normal allelomorph.

are not; therefore, the data reproduced from the table are accepted as being correct.

#### ANALYSIS OF THE DATA

In the first place, the data were analysed statistically to see that the eight different samples were in agreement with one another. The two parts of Table I, namely (a) the division at which segregation was observed to occur, and (b) the six arrangements of the ascospores in the asci, were each treated as contingency tables and the heterogeneity  $\chi^2$  calculated for each. The heterogeneity  $\chi^2$  for the segregation division was found to be 2.034 for 7 degrees of freedom; thus  $P$  is greater than 0.90, indicating that there is no heterogeneity. The heterogeneity  $\chi^2$  for the ascospore arrangements was 49.287 for 35 degrees of freedom; thus  $P$  lies between 0.10 and 0.20, again indicating that the data are homogeneous.

In those asci showing a first-division segregation two arrangements are observed. One shows the four dark spores in the upper four positions, *a* to *d*, in the ascus (type 1), the other shows the four dark spores in the lower four positions (type 2). In Fig. 2 are shown diagrammatically two types of bivalent that would give a first-division segregation for dark (+) versus light (r)

ascospores. When a bivalent of either of these types is oriented on the first-division spindle so that the + -bearing chromosome is directed towards the top of the ascus, a type 1 ascospore arrangement would result. Similarly, when the orientation is such that the + -bearing chromosome is directed towards the base

TABLE I

*Zickler's Data for Ascospore Arrangements segregating for Dark and Light Spore Colour in Bombardia lunata, normal × rubiginosa*

| Number of asci. | Segregation at |          | Arrangement of ascospores (cf. Fig. 1). |      |      |      |      |      |
|-----------------|----------------|----------|---|------|------|------|------|------|
|                 | Div. I.        | Div. II. | 1                                       | 2    | 3    | 4    | 5    | 6    |
| 1120            | 400            | 720      | 216                                     | 184  | 196  | 199  | 182  | 144  |
| 954             | 343            | 611      | 194                                     | 149  | 164  | 145  | 152  | 150  |
| 1028            | 365            | 663      | 205                                     | 160  | 193  | 155  | 170  | 145  |
| 1062            | 372            | 690      | 215                                     | 157  | 193  | 156  | 179  | 162  |
| 1046            | 372            | 674      | 220                                     | 152  | 197  | 146  | 180  | 151  |
| 999             | 375            | 624      | 216                                     | 159  | 196  | 136  | 140  | 151  |
| 981             | 359            | 622      | 212                                     | 147  | 172  | 159  | 154  | 137  |
| 1014            | 372            | 642      | 209                                     | 163  | 207  | 150  | 151  | 134  |
| 8204            | 2958           | 5246     | 1687                                    | 1271 | 1518 | 1246 | 1308 | 1174 |

TABLE II

*Correlated Orientation on the Two Second-division Spindles in the Asci of Bombardia lunata, normal × rubiginosa (cf. Fig. 1)*

| Ascospore arrangement.              |  | 3    | 4    | 5    | 6    |
|-------------------------------------|--|------|------|------|------|
| Observed                            |  | 1518 | 1246 | 1308 | 1174 |
| Expected, in absence of correlation |  | 1450 | 1178 | 1376 | 1242 |
| d                                   |  | +68  | +68  | -68  | -68  |

TABLE III

*Zickler's Data for Ascospore Arrangements segregating for Dark and Light Spore Colour in Bombardia lunata, normal × lactea*

| Number of asci. | Segregation at |          | Arrangement of ascospores (cf. Fig. 1). |     |     |     |     |     |
|-----------------|----------------|----------|---|-----|-----|-----|-----|-----|
|                 | Div. I.        | Div. II. | 1                                       | 2   | 3   | 4   | 5   | 6   |
| 2353            | 792            | 1561     | 428                                     | 364 | 421 | 370 | 414 | 356 |

of the ascus, a type 2 ascospore arrangement would result. *A priori*, we should expect that the direction of orientation of such a bivalent would be a matter of chance, and therefore that, on an average, each method of orientation would occur equally frequently. Therefore, unless there is some bias towards one orientation rather than the other, the two ascospore arrangements, types 1 and 2 respectively, should be equally frequent. On the contrary, the data show a considerable excess of the type 1 arrangement with the four dark ascospores in the upper positions. The probability that the numbers are equal is much less than 0.001;  $\chi^2$  is 58.5 for 1 degree of freedom. There is thus no doubt that the

possession of the normal allelomorph of rubiginosa or, perhaps more probably, of something linked with it, by one chromosome of the bivalent, biases the orientation of the bivalent so that the  $+$ -bearing chromosome is directed towards the top of the ascus.

The same bias appears at the second division, since the four ascospore

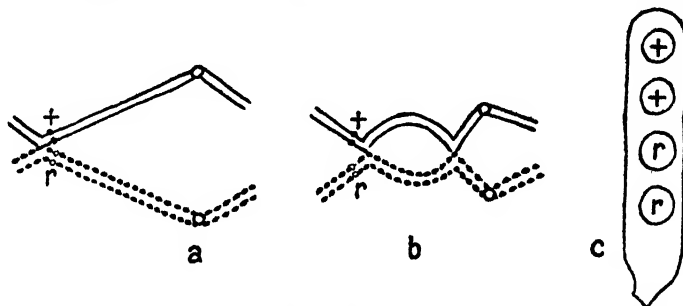


FIG. 2. Diagrams of possible bivalents at metaphase I that could yield a type 1 ascospore arrangement; (a) shows no chiasma between the centromere and the locus of rubiginosa ( $r$ ); (b) shows two complementary chiasmata between the centromere and the rubiginosa locus; (c) shows the telophase II distribution of rubiginosa and its normal allelomorph. The rubiginosa gene is shown as a small circle and its normal allelomorph as a black dot.

arrangements possible in the second-division segregation depart significantly from the equal frequency expected from a random orientation and segregation of the two chromatids of the chromosomes concerned. The probability that the observed frequencies form a bad sample of a population in which the four types occur equally often is much less than 0.001;  $\chi^2$  is 50.2 for 3 degrees of freedom.

In Fig. 3 is shown diagrammatically the way in which the four second-division ascospore arrangements arise. The manner of orientation of the bivalent on the first-division spindle at metaphase is immaterial since, in any case, each of the telophase I nuclei would contain a chromosome having  $+$  borne on one chromatid and  $r$  on the other. On the second-division spindles the orientation of these chromosomes is biased so that the  $+$ -bearing chromatid is directed towards the top of the ascus more often than would be expected if the orientation were purely a matter of chance.

It should be noted that the biases observed at the second division appear to be different from one another. Thus the bias on the lower spindle is slightly greater than the bias on the upper spindle. The probability of segregation to an upper position is  $0.5387 \pm 0.0069$  for the lower spindle as against  $0.5131 \pm 0.0069$  for the upper spindle. Further, both second-division biases are less than the first-division bias; at the first division the probability of segregation to an upper position is  $0.5703 \pm 0.0091$ . The first-division and the second-division lower-spindle biases are clearly significant, but the evidence that there is a bias on the second-division upper spindle is not significant. Are these biases and probabilities actually different from one another? The proportions of upper and lower segregations for each may be compared in a  $3 \times 2$  con-

tingency table. This gives a  $\chi^2$  of 14.57 for two degrees of freedom and shows that the probability that the biases are equal is less than 0.001. The bias on the first-division spindle is clearly greater than either of the second-division biases. The two second-division biases are also significantly different; a contingency  $\chi^2$  gives 6.86 for 1 degree of freedom, so that  $P$  is just less than 0.01.

The next question is whether the biases on the upper and lower second-division spindles act independently of one another. We may calculate the

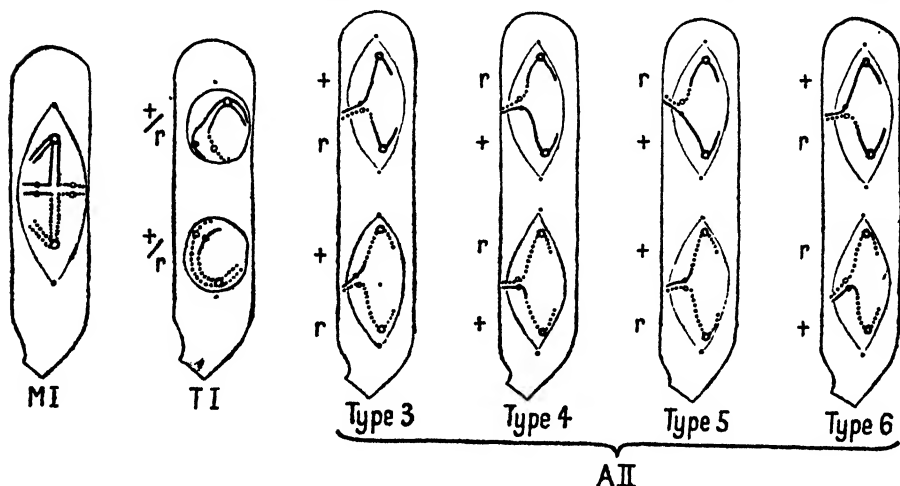


FIG. 3. Diagram to illustrate the origin of asci of types 3 to 6. A rubiginosa bivalent lies on the metaphase I spindle; it has one chiasma between the centromere and the rubiginosa locus. The rubiginosa gene is shown as a small circle and its normal allelomorph as a black dot. The resulting telophase I and anaphase II distributions are shown.

expected numbers of asci of types 3 to 6 on this assumption by dividing the 5,246 asci amongst the four types in the respective proportions of  $0.5387 \times 0.5131$ ;  $0.4613 \times 0.4869$ ;  $0.5387 \times 0.4869$ ;  $0.4613 \times 0.5131$ . This gives an expectation of 1,450, 1,178, 1,376, and 1,242 for the four classes of asci. The observed numbers (cf. Table II) differ significantly from these;  $\chi^2$  is 14.2 for 3 degrees of freedom, so  $P$  is less than 0.01. We may infer that the biases on the two second-division spindles do not operate independently. Moreover, the deviation from expectation is in the direction of a *correlation* between the orientation of the affected chromosomes on the two second-division spindles. The  $+$ -bearing chromatids are correlated in the spindle pole, upper or lower, to which they are segregated.

We may measure the bias by which the  $+$ -bearing chromosome is segregated to the upper pole of a spindle as the percentage excess of  $+$ -bearing chromosomes which reach the upper pole. This may very slightly underestimate the bias on the first-division spindle for the following reason. If the bias acts irrespectively of the number and relationship of any chiasmata between the centromere and the locus of the biased gene, then a bivalent with a four-strand double cross-over (two complementary cross-overs) between the

centromere and the + locus would behave so that the segregation of the + -bearing chromosome was biased towards the lower spindle pole. Unfortunately, we have no adequate means of estimating the number of four-strand doubles in our present sample.

If we compare the apparent first- and second-division lower and upper biases, respectively  $14.06 \pm 1.82$  per cent.,  $7.74 \pm 1.28$  per cent., and  $2.62 \pm 1.34$  per cent., we notice that the first-division bias is twice that at the lower second division. This fact may give some help in exploring the possible mechanism of action of the bias, but in the absence of any other evidence pointing the way, any speculations are likely to be unfruitful.

Before proceeding to discuss the general problem of polarized or preferential segregation, we should also consider similar, but more limited, data that Zickler has given for segregation of lactea, the other pale ascospore mutant, from the normal (+ or viridis) type. The data are reproduced in Table III. Zickler's total of asci analysed, given in the text as 2,355, seems to be in error. The ascospore arrangements show that in this case, also, there is a bias towards an upper position in the segregation of + from lactea. The probability that the two first-division arrangements (types 1 and 2) are equal is just greater than 0.02 ( $\chi^2$  is 5.18 for 1 degree of freedom). The probability that the four second-division arrangements (types 3 to 6) are equal is just less than 0.05 ( $\chi^2$  is 7.93 for 3 degrees of freedom). The probability that the departure from equality, at the first and second divisions together, would be as great as that observed is just greater than 0.01 ( $\chi^2$  is 13.1 for 4 degrees of freedom). There is evidence, though not highly significant, that the segregation of lactea from + is also biased. Moreover, it appears that the magnitude of the bias in the case of lactea is the same as in the case of rubiginosa. The probability that the first-division biases are alike is 0.10 to 0.20 ( $\chi^2$  is 2.27 for 1 degree of freedom), while the probability that the second-division biases are alike is 0.30 to 0.50 ( $\chi^2$  is 2.8 for 3 degrees of freedom). The two sets of data are in good agreement. The reason for the agreement may be that lactea and rubiginosa are allelomorphs linked to the same biasing material in the same chromosome. Some doubt is thrown on this possibility by the fact that the proportion of first- and second-division segregation is not for certain the same in +/lactea as in +/rubiginosa. A contingency test shows a  $\chi^2$  of 4.62 for 1 degree of freedom, so that *P* lies between 0.02 and 0.05, though nearer the latter figure. Rubiginosa is certainly in the sex chromosome, but the data for lactea are too few to be conclusive. It should be recalled that lactea usually arose from rubiginosa as spontaneous sectors. This suggests that there is a definite connexion between the two mutants.

## DISCUSSION

We have established beyond question that in Zickler's data there is a bias for the dark-spored genotypes to occupy upper positions after the first and second divisions of meiosis. It might be thought that the bias was on the part

of the observer, acting in some way so that dark spores in the upper part of the ascus attract greater attention than when they occupy the lower part. The similar biases for the asci of normal  $\times$  rubiginosa and of normal  $\times$  lactea would be explicable on such a basis. It is difficult, however, to see how any bias on the part of the observer would account for the inequality and correlation of the biases in the upper and lower halves of the asci showing second-division segregation. In the absence of any evidence to the contrary we may assume that the bias is natural to the material and dependent on a biased orientation of the bivalents and chromosomes concerned.

It is one thing to demonstrate the existence of a phenomenon, but quite a different matter to show how it is brought about. In the present case, how does the tail wag the dog? How does the gene locus direct and influence the centromere, the body normally responsible for orientation and movement of the chromosome on the spindle? At first sight the behaviour seems contrary to the principle that the behaviour of the chromosomes is subject to the joint action of the genotype of the whole nucleus. We may need to revise and modify that hypothesis.

The present case emphasizes the principle that any polarized segregation involves a dual inequality. One is the difference possessed by the pair of chromosomes exhibiting the bias. The other is the difference that must exist between the two ends of the cell in which the bias is exhibited. In *Bombardia* there must be some difference between the base and apex of the ascus or more exactly a gradient from the base to the apex. This is strikingly confirmed by the fact that the bias on the second-division lower spindle is higher than the bias on the second-division upper spindle, where it is only slight. Taken in conjunction with the facts that the biases on both the second-division spindles are lower than the one on the first-division spindle and that between the two divisions the ascus will have grown, physiologically if not in stature, we may infer, for instance, the diffusion of some substance from the base of the ascus. It appears, too, that this substance is used up in the course of the division so that the gradient between the poles of the second-division upper spindle is slight compared with that between the poles of the second-division lower spindle and both are much smaller than the first-division gradient. Alternatively, uniform production in the ascus accompanied by diffusion into the mycelium would also provide a suitable gradient.

We may further suppose that a gradient of a certain steepness in a proportion ( $p$ ) of asci causes those lower spindles that would otherwise have segregated dark to a basal position to invert, and that a gradient of a higher steepness in a proportion ( $q$ ) of asci causes similar lower and upper spindles to invert. Thus the expected random orientation ratio of types 3, 4, 5, and 6 asci to one another would be converted from 0.25:0.25:0.25:0.25 into

$$0.25 + p + 3q : 0.25 - p - q : 0.25 + p - q : 0.25 - p - q.$$

A rough evaluation suggests both  $p$  and  $q$  are about 0.01. The expected numbers of the four ascus types would be 1,521.5, 1,206.5, 1,311.5, and



1,206.5, forming a good agreement with observation. This hypothesis accounts for the correlation noted on page 125. It is rather idle to speculate further in this direction, but it is obvious that possibilities for some interesting experimental work are opened up.

It is quite possibly not the rubiginosa, or lactea, locus itself that is responsible for the bias. Instead it may be something to which the affected locus is linked, the affected loci merely acting as convenient markers of the biased chromosome. Some other cases of preferential or polarized segregation have been reported in the literature, and these show more or less conclusively that the effect is dependent upon the heterochromatin regions of the chromosomes.

Sturtevant (1936) reported cases of preferential segregation in *Drosophila melanogaster* in respect of the fourth chromosome. Segregation in triplo-IV females usually gave two chromosomes to one pole and one to the other. The three resulting types of segregation, however, did not usually occur with equal frequencies. A number of different IV chromosomes were studied and were found to be capable of arrangement in a definite seriation, such that any chromosome would, in a triplo-IV female, pass to the haplo-IV pole more often than would any chromosome lying above it in the series. This was found to hold provided the two chromosomes were tested in similar experiments or in the same experiment.

If two IV chromosomes, *A* and *B*, were tested against any third IV chromosome, *C*, then in those cases in which *A* and *B* separated, *C* would go with *A* in a proportion of the cases,  $r$ , that was independent of the nature of *C*. Thus the proportion,  $r$ , was a constant property of the pair *AB*.

The segregation in triplo-IV males was also non-random. It deviated from randomness in the same direction as that in corresponding female, but less markedly.

Sturtevant dealt with a large number of different IV chromosomes, some of which had arisen from others in known ways. A study of the genetic relations of these and of their preference properties showed that no one locus was responsible for the preference properties. The evidence pointed to the neighbourhood of the centromere as being the most important part of the IV chromosome in determining preference. This region, as well as the end of the chromosome, consists of inert material (heterochromatin) according to Bridges (1935).

Rhoades (1942) has recently described a clear case of preferential segregation in maize. An extra piece of chromatin (apparently heterochromatin in character) was attached to the long arm of chromosome 10 in the strain that behaved exceptionally. In maize the functional megaspore giving the embryo-sac is the chalazal one. In plants heterozygous for the abnormal chromosome 10 a majority of the chalazal megaspores receive the abnormal chromosome. This means that the orientation of the chromosome 10 bivalent is biased. It happens that the gene *R*, one of the major genes responsible, among other effects, for the development of anthocyanin in the aleurone layer of the endo-

sperm, is located close to the end of the long arm of chromosome 10. Therefore, *R* is very closely linked to the extra piece of chromatin which is preferentially segregated to the chalazal (functional) megaspore. Hence a majority of the embryo-sacs in the cross, *R* abnormal chromosome 10/*r* normal chromosome 10, receive *R* with the abnormal chromosome. Contrariwise, the abnormal chromosome 10 reduces the rate of pollen-tube growth, so in the above cross a minority of the functional pollen-grains would carry *R*. Thus, there is a relative excess of *R* amongst the eggs and a deficiency of *R* among the sperm.

Thus, in maize, the possession of an extra piece of heterochromatin leads to non-random orientation of the bivalent in which it occurs. In *Drosophila* different IV chromosomes show characteristic preference segregations in triplo-IV flies, and these preferences are dependent upon differences in the heterochromatin regions of the different chromosomes. It appears, then, that hybridity in the heterochromatic regions may disturb the random orientation of a pair of chromosomes at meiosis. Moreover, this effect appears to be specific to the bivalent hybrid for the heterochromatin difference and not to affect other bivalents of the chromosome set.

There is no evidence for a heterochromatin difference between the rubiginosa and normal strains of *Bombardia lunata*. Zickler originally isolated a number of wild type strains of the fungus and rubiginosa arose spontaneously from one of these; lactea arose from rubiginosa. The fact that the fungus is heterothallic implies outbreeding and therefore a considerable amount of hybridity. The 'sex chromosomes', those bearing the 'plus' and 'minus' factors, may well accumulate other differences, and it is clear that the rubiginosa locus is on the sex chromosomes. There is 37.4 per cent. crossing-over between the sex genes and the rubiginosa locus. It would be interesting to know the behaviour of the rubiginosa locus when tested against further different wild type chromosomes.

It should be stated that the data of Goldschmidt and Kaksuki (1931) and Goldschmidt (1932) showing a correlated segregation for a sex-linked and an autosomal gene in *Bombyx mori* do not seem to be capable of explanation as a case of polarized segregation.

#### SUMMARY

In *Bombardia lunata* there are two mutants, rubiginosa and lactea, which have light-coloured, transparent ascospores, the normal ones being dark. Segregation for ascospore colour in a cross between light and dark strains is shown directly in the asci, which have an orderly row of eight spores.

Zickler's (1934) data have been re-examined and found to show biased segregation of the mutant genes from their normal allelomorphs, such that the normal allelomorphs tend to be segregated to the upper spindle poles at the first and second meiotic divisions. The bias at the first division is about 14 per cent., that on the second-division lower spindle 7.7 per cent., and that on the second-division upper spindle 2.6 per cent.

There is no evidence, as there was in previously reported cases of non-random segregation, whether hybridity of the heterochromatic regions of the affected bivalent is responsible for the bias. The bias must depend upon a difference, probably a graded difference, between the upper and lower regions of the ascus, as well as upon a difference between the biased chromosomes.

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# The Germination of the Seed of *Striga lutea*

## I. Host Influence and the Progress of Germination

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With eight Figures in the Text

### INTRODUCTION

*STRIGA LUTEA* is an angiospermous parasite belonging to the Rhinanthoideae. It attacks the roots of a variety of species of which maize, sorghum, and sugar-cane are economically the most important. The germination of this species is similar to that of certain other allied parasites. Fuller (1900), Pearson (1911, 1912), Saunders (1933), and Kumar and Solomon (1940) have all found that the seed will only germinate when in close proximity to a host root, and they attribute this effect to the secretion by the root of a particular stimulant. A similar condition has been observed with *Orobanch*e (Koch, 1887; Tate, 1925; Barcinsky, 1934; Chabrolin, 1934), *Lathraea* (Heinricher, 1894, 1898, and Tozzia (Heinricher, 1901).

The present investigation is designed to amplify the results of earlier workers in one important respect. While the host effect is clearly a dominating feature of the situation, it requires to be related to the complex of factors that control germination. Accordingly, in the present series of experiments certain quantitative aspects have been examined that indicate the extent to which the host effect limits the whole process. The results are presented in another section; but it is necessary to consider here certain other preliminary observations that form the basis of the techniques that have been elaborated for the main purpose of the investigation.

### PRELIMINARY OBSERVATIONS

The majority of seeds of *Striga lutea* when exposed to the conditions of temperature and moisture in which germination usually occurs do not develop. In these circumstances they may remain for months in an apparently dormant condition. The radicle does not emerge and the cotyledons do not expand. Nevertheless the seeds do absorb water and the testa may rupture as a result. On the other hand, when in addition to being moistened and exposed to a high temperature they are placed in proximity to a host root, then the

seeds ultimately germinate in the usual manner. This may be demonstrated by the method used by Kumar and Solomon (1940). The parasite seeds are sprinkled over the surface of moist filter-paper lying in a Petri dish. One or two host seeds are added to the culture and the whole incubated. Host roots develop over the surface of the filter-paper and along the course of these germinated parasite seeds may in due time be observed.

But even then germination is neither rapid nor uniform; it is sporadic and may extend over a period of weeks. This condition makes it extremely difficult to measure the germination rate in the presence of the host roots. Reproducible results can only be obtained when all the experimental conditions are controlled, and with a composite culture involving a host plant and parasite seeds, this requirement is difficult to fulfil when the experimental period must necessarily be prolonged. Fortunately it has been found that the difficulty can be circumvented by a comparatively simple provision. Germination is both rapid and uniform when the seeds are exposed for a week or more to moisture and a high temperature before they are exposed to the influence of the host factor. This observation not only facilitates considerably the measurement of germination but it is also of some significance in relation to the interpretation of the sequence of events that occur in the development of the seed. Two terms are used in this paper in connexion with this phenomenon. The term 'pretreatment' is applied to the process of inducing enhanced germination before stimulation with the host factor, and that of 'germination capacity' to the germination that may be induced by the application of the host factor.

The reports of earlier investigators suggest that among parasites showing host-dependent germination the phenomenon of slow and sporadic germination is not peculiar to *Striga lutea*. It is mentioned by Köch (1887) in relation to Orobanche, by Heinricher (1894) in relation to Lathraea; and Chabrolin (1938), who also comments on the same phenomenon in Orobanche, makes the significant statement that germination is accelerated if the seeds are kept moist for nine days before the application of the host factor.

The fact that in a composite culture germination is restricted entirely to the immediate vicinity of the host root suggests strongly the operation of a chemical stimulant secreted from the host, but it does not exclude other possibilities. The conclusive evidence is provided by the induction of germination with fluids that have been in contact with the host root but which have been separated from it. Saunders (1933) finds that seeds of *Striga lutea* moistened with water that has been passed through sand in which host roots are growing germinate, whereas others moistened with pure water do not. The experiments of this last worker have been repeated in this laboratory without conspicuous success, the different result being undoubtedly due to the different climatic conditions in which the experiments were conducted. The work of Saunders was done in South Africa, that of the present investigators in Manchester. Another technique more appropriate to English conditions has been devised, which also provides conclusive evidence of the

incidence of a chemical stimulant. Host seeds are floated on water and allowed to germinate in the dark in that position. The roots develop freely in the water, and this when it is separated from the host roots and applied to suitably treated seeds stimulates them to germinate.

Earlier workers have stated that germination occurs only under the influence of the host stimulus. The preliminary results in this investigation leave little doubt that percentage germination is very considerably enhanced when the host factor is applied, but observations made on numerous control cultures in which the seeds are moistened only with water show that some seeds, though a very small proportion, germinate without this stimulus, the proportion being also much smaller than with the host stimulus. This independent germination may be a factor of some importance in the general economy of the species. It is of some interest to notice that Barcinsky (1934), working with *Orobanche*, and Chemin (1925), working with *Lathraea*, both find that although germination is highest with the host factor, nevertheless some occurs in the absence of the stimulus.

#### MATERIALS AND METHODS

Two methods have been devised for measuring the germination rate, each suggested by one of the two situations in which germination may be observed.

The first is designed to provide data on germination in the presence of the host root, particularly with reference to the rate of germination at determined distances from it. The experimental arrangement of Kumar cannot be adapted for this purpose. When the culture is established in a Petri dish, it is difficult to maintain constant moisture conditions, and it is difficult to make elaborate microscopical observations at known distances from the host root. Since the seeds are minute ( $0.30 \times 0.18$  mm.), whatever the arrangement adopted it must be such that the surface over which the seeds are distributed is accessible to microscopical examination. The technique finally adopted involves culturing both the seeds and the host root between moist filter-paper and the inner surface of a glass cylinder, open at both ends, and the outer surface of which is covered with celluloid ruled into squares, the length of whose sides is 2 mm. The system is vertical and stands in a small Petri dish containing water. The water is drawn upwards by the filter-paper and distributed over a constant vertical length. Thus in different cultures at corresponding heights the level of the humidity factor is the same. The parasite seeds are sprinkled on the filter-paper before this is applied to the glass; the host roots arise from seeds lying along the upper rim of the cylinder in a groove formed by drawing the wet filter-paper a short distance away from the glass. All cultures are maintained in the dark in an incubator in which the temperature is kept constant at  $35^{\circ}$  C.

Germination is measured with the cylinder placed lengthwise on the stage of a microscope. In this position the seeds and the host root are seen through the celluloid covering of the cylinder. The grid ruled on the celluloid provides the means of measuring distances from the host root and of determining

the relation between germination and distance. As seen through the microscope the host root traverses successive squares in a more or less vertical series (when the cylinder is in the normal position); and each of the root-occupied squares is a member of a horizontal series that traverses the circumference of the cylinder. In this series the vertical axes of successive squares are separated from that of the root-occupied square by 2, 4, 6, 8 mm., and so on. Thus, if the square traversed by the host root is taken to represent 0 mm. from the host root, that adjacent to it represents 2 mm., the third in the series 4 mm., and the fourth 6 mm. The series of squares representing determined distances is of course repeated on the two sides of the root-occupied square. The series is limited to three on either side of the root-occupied square, and in any horizontal series there are seven sets of observations. But the root traverses successive squares vertically and thus the horizontal series of observations may be repeated at intervals of 2 mm. along the vertical axis. The measurements are not usually made along the whole length of the cylinder. No observations are taken immediately below the upper rim or immediately above the lower. The observations for each square are recorded separately, and thus the data can be grouped in different ways according to the purpose for which they are required.

Observations are made at intervals of 24 hours and over a period of 5 days. Since each square can be identified by the position in the vertical series, each measurement can be related to the one made in the same square on the previous occasion.

The second method is based on the fact that germination can be induced with solutions obtained from the host root. This fact provides the possibility of examining the different phases of the host-parasite relationship separately and of investigating certain aspects of the situation which cannot be analysed when the host root and the parasite seed are cultured together. If the variables involved in the production of the host-factor fluid and in the germination of the seed can be controlled, then it should be possible to examine the germination of the seed with a solution of standard activity and to investigate the conditions for the production of the host factor by using seed of standard germination capacity. In the method described below an attempt has been made to secure the necessary measure of control.

The method involves two techniques, one for the production of the active fluid and another for determining the germination reaction of the seed. It is extremely doubtful, even with favourable climatic conditions, whether the method used by Saunders (1933) can be adopted for obtaining solutions with a standard activity. When water is percolated over a root system growing in sand the extent of that root system is unknown, and it is probably highly variable even for plants of the same age. In the technique used here the vessel in which the seedlings are grown is a large boiling-tube with a capacity of 60 c.c. This is supplied with 20 c.c. of distilled water and then rested at an angle of 30° to the horizontal, giving a long but narrow water surface. The seedlings are arranged on a float, consisting of a short length of thin-

walled glass tubing, closed at both ends, bent into the form of a narrow U, and with the two arms having a gap between them which is less than the width of the seeds. Ten seeds, selected from a group germinating in a Petri dish for two days and all at approximately the same stage of development, are transferred to a float which is immediately placed on the surface of the water in the sloped boiling-tube. When the seedlings are in position the mouth of the boiling-tube is closed with a rubber stopper. Clearly in this arrangement the conditions of culture can be controlled and can be varied according to requirements. In the present series of experiments the technique has been designed to produce a solution of standard activity, and the cultures have been continued for a standard period of five days, in the dark and in a chamber in which the temperature has been maintained constant at 22° C.

For the complementary technique for studying germination the normal Petri-dish method is unsatisfactory, since the continual evaporation that occurs from these dishes makes it difficult to control either the concentration or the volume of fluid in contact with the seeds. An adaptation of the normal hanging-drop technique has been used which gives more consistent results. A group of seeds is placed on a cover slip together with a standard volume of fluid. In the usual way the cover slip is inverted and secured to a glass ring attached to a microscope slide. The cover slip is attached to the ring and this to the slide with a mixture of vaseline and beeswax. The culture is examined microscopically after incubation at a constant temperature. With this method a certain amount of evaporation into the confined space below the drop no doubt occurs, but the space is small and the reduction in the volume of the drop is negligible. Moreover, the rings being of standard size, the volume they each enclose is constant, and the volume of the drop is therefore reduced by a constant amount. In this arrangement the conditions of the test can be varied according to requirements, but for standard purposes it has been found that adequate results are obtained by using 0.01 c.c. of host fluid in the hanging drop and incubating at 35° C. for 24 hours. The ideal arrangement would be one in which not only the volume of liquid but also the number of seeds is controlled. Unfortunately it has not hitherto been possible to secure this condition. For reasons already explained it is necessary to keep the seeds moist for some time before they are exposed to the host stimulus; also the germination rate is very markedly reduced if the seeds are subjected to any drying while being transferred to the hanging-drop chamber from the dish in which they are kept moist. Thus it has been found necessary to remove on the point of a needle a mass comprising a variable number of seeds, rest this on filter-paper for a few seconds to remove any free liquid, and then immediately to transfer it to the cover slip; the number of seeds thus carried varies between 25 and 50. Fortunately experiments in which the seed number has been deliberately varied over a wide range have shown that with 0.01 c.c. of host fluid the seed number can be varied from 7 to 80 without significantly affecting percentage germination.



Two important precautions must be observed in this technique. All glassware with which the seeds are brought in contact must be very carefully cleaned to remove any traces of grease, and the time occupied in preparing the cultures must be standardized and be as short as possible.

Each germination test in this series involves six replicates, and each of the values obtained by the use of this technique is the mean of six observations. Each set of replications with the host fluid is accompanied by the same number of controls in which the fluid is water. The large number of controls is necessitated by the fact that independent germination may occur.

A single host species, *Sorghum vulgare*, has been used throughout. This plant is particularly suitable, since the seedlings are small and easily manipulated, and the parasite reacts to it more readily than it does to maize. The sample of parasite seed was obtained from the Assistant Director of Agriculture in Nyasaland, to whom we are deeply indebted for his generous help.

Most of the results given in this paper have been obtained from composite cultures on cylinders, and they show the effects on germination of certain conditions that are established after exposure to the host root. But since germination is also affected by conditions applied before host stimulation, the presentation of the main body of results is preceded by the description of the results of experiments designed to elucidate the nature of the 'pretreatment' effect.

#### EXPERIMENTAL RESULTS

*The effect of pretreatment.* The treatment involves exposing the parasite seeds to moisture and a warm atmosphere, and the effects of two variables in this process, temperature and period of exposure, have been examined both in composite culture and in hanging-drop cultures.

Two groups of cylinders were prepared in the usual way with seeds of the parasite in position and the filter-paper moist, but without host seeds along the upper rim. One group was transferred to a chamber in which the temperature was maintained constant at 22° C., and another to an incubator in which the temperature was at 35° C. At intervals of two days one cylinder was removed from each group, host seeds were planted along the upper rim, and both incubated at 35° C. for five days. At the end of this time determinations of percentage germination were made within a representative distance of 4 mm. along the host root. The results are shown in Fig. 1, in which each value represents observations along a single root.

The matter was further investigated by germinating the pretreated seeds with a standard host solution. The seeds were in this case sprinkled on wet filter-paper in Petri dishes, dishes being kept at temperatures of 22°, 30°, and 35° C. Samples were taken from each dish at intervals of three days and transferred to hanging-drop chambers incubated at 35° C. The results are shown in Fig. 2.

Figs. 1 and 2 suggest that as the length of the pretreatment period

increases so does the germination capacity of the seed, until a certain optimal exposure time is reached, after which the germination capacity falls. Further, at 22° C. the germination capacity tends to rise more slowly than it does at

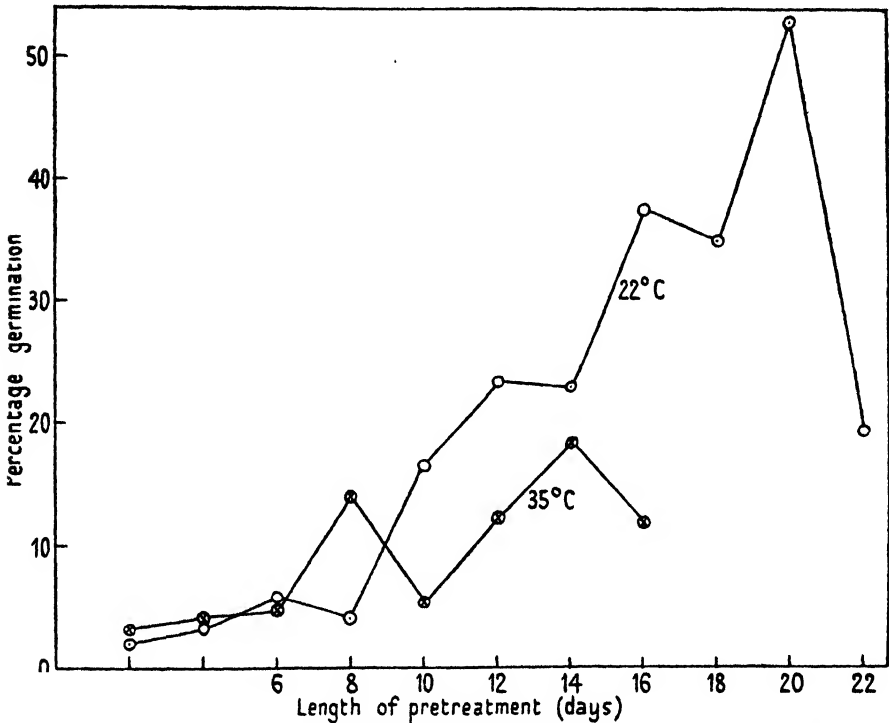


FIG. 1. Effects of length and of temperature (22° C. and 35° C.) of pretreatment on germination capacity of parasite seeds. Observations made with composite cultures on cylinders. For details see text.

the higher temperature, but eventually it reaches the same maximum value as at 30° C. At 35° C. the rise in germination capacity is extremely rapid.

The falling germination capacity with prolonged pretreatment is more strongly emphasized by Fig. 3. The data of this series were obtained in the same way as those of Fig. 2. In Fig. 3 an additional set of data is also included, derived from observations in control cultures in which no host fluid was provided. The seeds for the control culture were taken from the same dish and at the same time as those cultured with the host solution; the pretreatment temperature to which the seed population was exposed was 22° C. At the time of removal the seeds had not germinated. Nevertheless when they were transferred to the hanging water drop and exposed to a temperature of 35° C. a few seeds germinated. In the earlier and later phases of pretreatment the capacity for independent germination is extremely low, but at the phase at which the highest germination is given by the host solution the number of

seeds that develop independently may reach 15 per cent. This phenomenon has been observed several times with the hanging-drop technique and no doubt occurred on the cylinders, but it could not be recognized there since

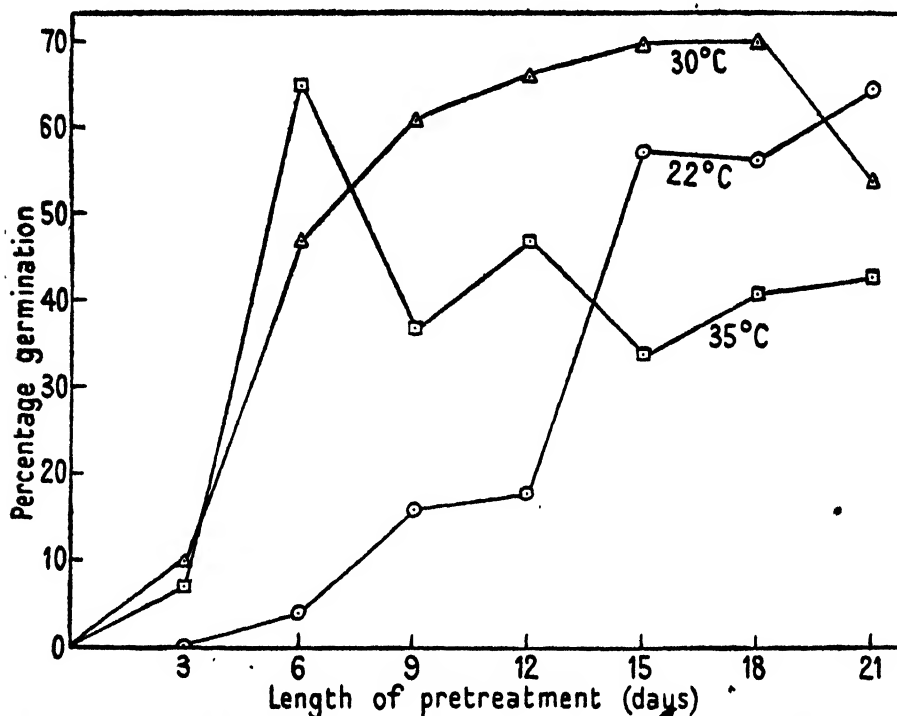


FIG. 2. Effects of length and of temperature (22° C., 30° C., and 35° C.) of pretreatment on germination capacity of parasite seeds. Observations made with host solution. For details see text.

germination at considerable distances from the host root may occur under the influence of extreme dilution of the host factor.

*Germination in the presence of a host root.* The data of this series were obtained with cylinders pretreated for 8 days at 35° C. Measurements of percentage germination were made at intervals of 24 hours over a period of 5 days in the manner already described. The observations at determined positions are grouped in various ways to show the effect on germination of (1) distance from the host root, (2) position with respect to height in the cylinder, (3) the development of lateral roots, and (4) the relative density of the distribution of the parasite seeds.

The germination frequency at 0, 2, 4, and 6 mm. from the host root is shown by the data of Fig. 4, which represent the means of the corresponding observations taken from seven different cylinders. It is evident that over the whole cylinder the germination frequency tends to be low during the first 24 hours, to rise sharply between 24 and 48 hours, and subsequently to

change only slightly, so that between 72 and 120 hours there is little if any increase in percentage germination. Further, a different limiting value for percentage germination is established for each representative distance, and

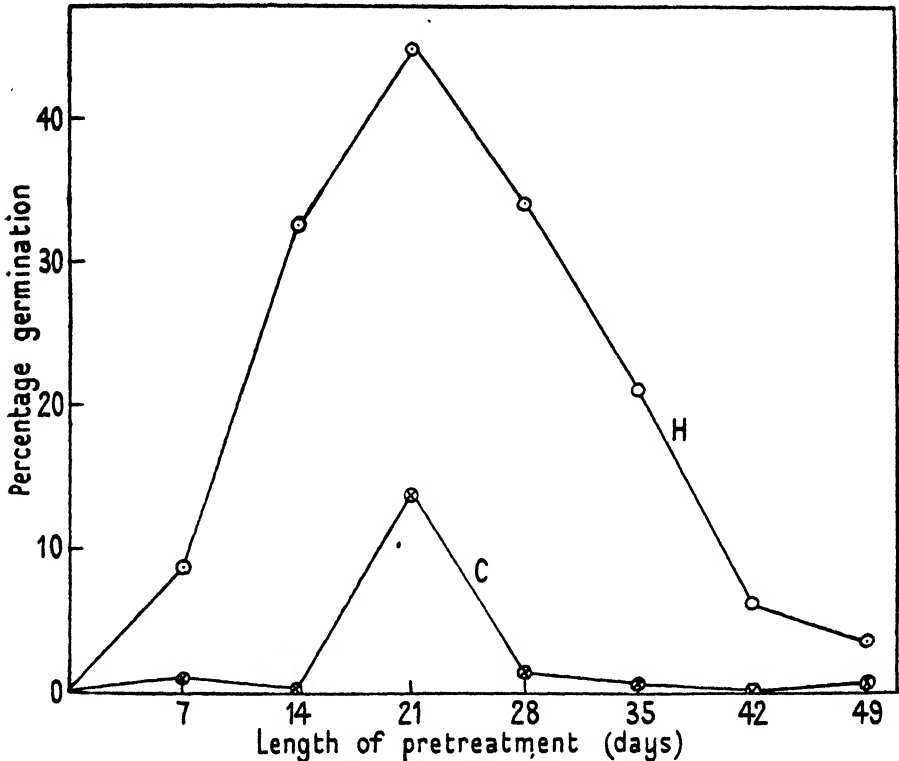


FIG. 3. Effect of length of pretreatment at 22° C. on germination capacity. Observations made with host solution (H), and with water in control cultures (C).

at 120 hours, when no further increase in percentage germination is occurring, the value for this quantity tends to decrease linearly with increasing distance from the host root.

The variation within any particular series of replicated experiments is considerable. Fig. 5a-c represents three of the separate sets of observations made on individual roots, taken from the series from which the data of Fig. 4 were obtained. The general features of each individual group are similar to those of the derivative series. The individual sets of observations, however, differ among themselves and from the derivative series in certain important respects. The values for corresponding distances vary greatly, the decrease with distance is not always linear, and the shapes of the curves are not uniform. The corresponding differences between any two series are highly consistent, and cannot therefore be attributed to errors of observation. The possibility that they are due to variable environmental conditions is excluded

by the conditions of the experimental design. It must therefore be concluded that the differences between the results given with different roots are the results of variables in the roots themselves. The factors that determine the

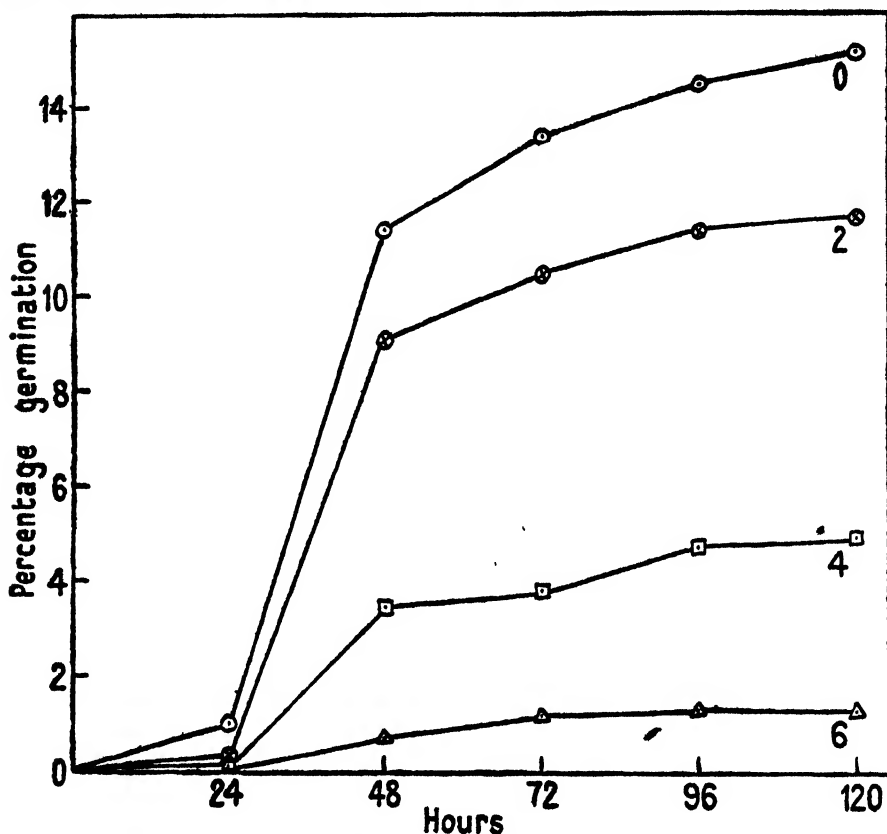


FIG. 4. Germination in composite cultures at various distances (0, 2, 4, and 6 mm.) from host root. Data represent means of 7 replicate cultures.

variable stimulating capacity of different root systems are no doubt complex, but one of them is undoubtedly the capacity to produce lateral roots.

Different roots differ considerably in this respect. The one with which the results of Fig. 5a were obtained produced abundant laterals; those from which the values of Fig. 5b and c were derived, did not. Fig. 5a shows a sudden increase in germination between 96 and 120 hours at 0 and 2 mm. from the host root; this is undoubtedly related to the comparatively high lateral root development. The effect of this variable may also be demonstrated by comparing the germination of seeds between 2 and 6 mm. from the host root in regions of the same root in which laterals do and do not arise. Such a comparison is shown by the data of Fig. 6. It is clear that the germination is highest in regions in which laterals are formed and that in these regions germination continues to increase over a relatively long period.

The difference between the values of Fig. 5*b* and 5*c* cannot be attributed to corresponding differences of branching. They are simply the result of different capacities for the production of the special host material.

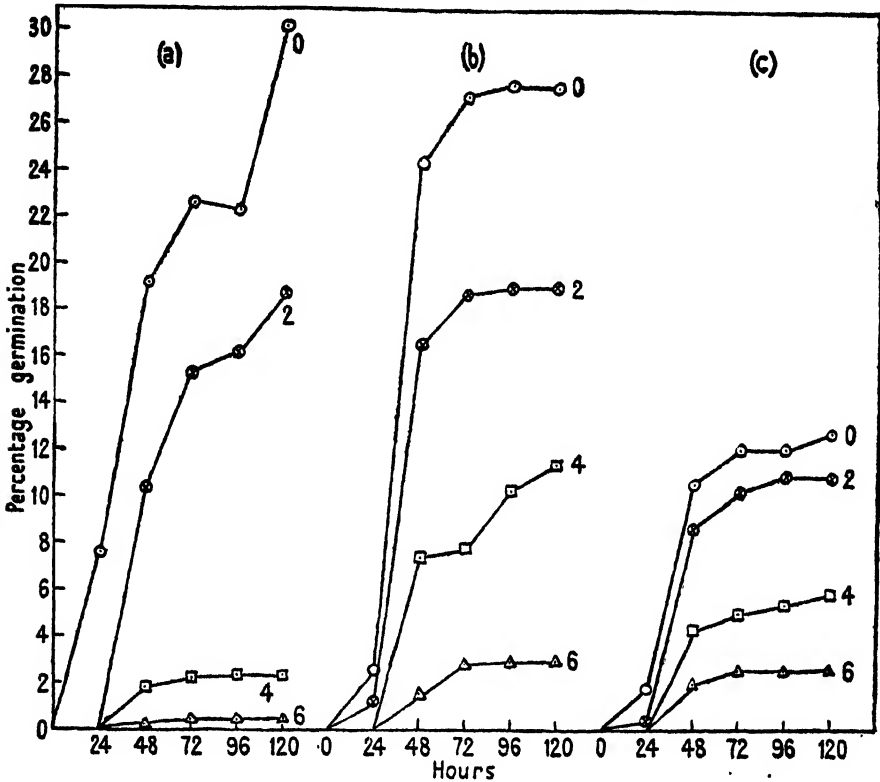


FIG. 5. Germination at various distances (0, 2, 4, and 6 mm.) from three individual roots.

The effects of two sets of variables in the cylinders are of some significance in relation to the data of Figs. 4 and 5. Since the root must grow downwards from the top of the cylinder, the conditions with respect to stimulation by the host are not uniform in all parts of the culture throughout the period of the experiment. The effect of this condition is shown by the data of Fig. 7, which were obtained by analysing the measurements on germination on successive days, within a distance of 4 mm. from a series of host roots, into groups corresponding to successive zones, each 10 mm. broad, along the vertical axis. Clearly, corresponding changes in germination frequency do not occur simultaneously throughout the length of the cylinder. At the end of 24 hours percentage germination is highest towards the upper end of the cylinder and decreases progressively towards the lower; whereas the maximum increase at the upper end of the cylinder occurs during the first 24 hours, in the lower regions of the cylinder the greatest increase occurs between 24 and 48 hours.

The second variable situation in each cylinder is the result of the method of applying the parasite seeds to the filter-paper. They are sprinkled on, with the result that wide local variations in seed density occur. This factor, if it

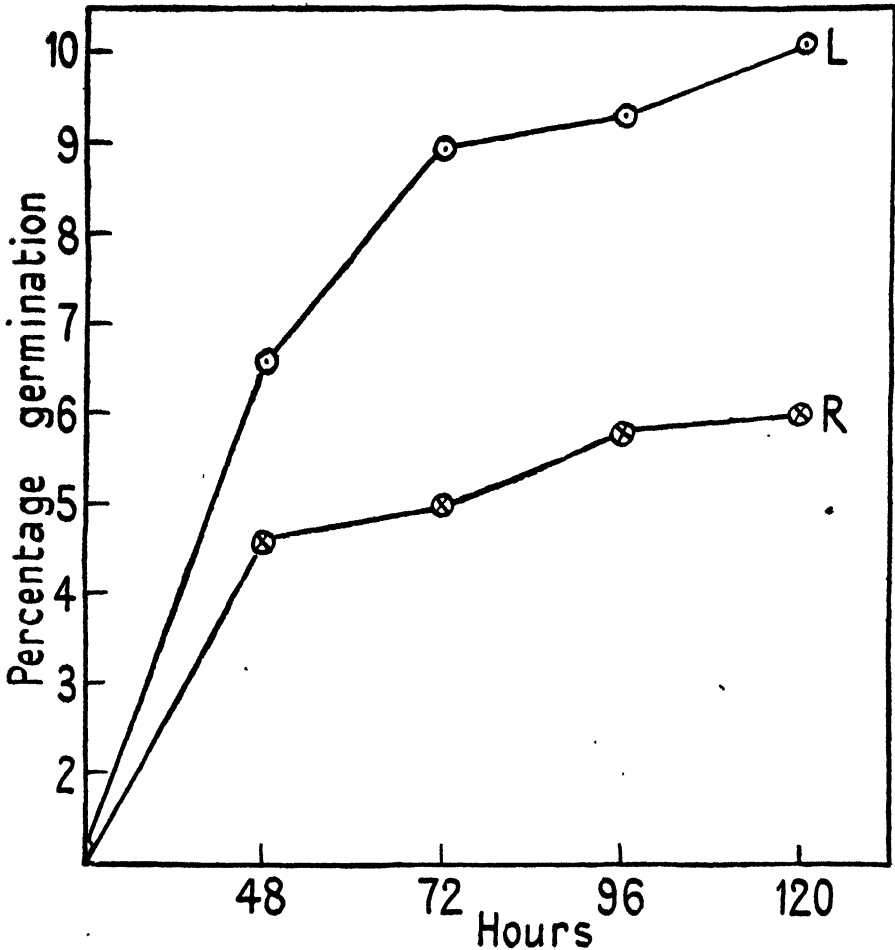


FIG. 6. Germination frequency between 2 and 6 mm. from a host root in regions occupied (L), and not occupied (R) by a lateral root.

influences germination, might affect the reaction of the seed to the host stimulus and thus affect the significance of the cylinder data presented above. The effect of this variable can be examined by classifying percentage germinations at the end of the experimental period and at representative distances from the host root into groups according to the total number of seeds originally observed in each 4 sq. mm. The results of such an analysis are shown in Fig. 8. The values given for the highest densities observed (20 seeds per sq. mm.) are of very doubtful significance, since these are rare and the values given are based on comparatively small samples. It is clear that seed density

in the conditions of the composite cultures has little effect on germination. With densities varying between 4 and 20 seeds per sq. mm., there may be slight differences but they are so small as to be negligible.

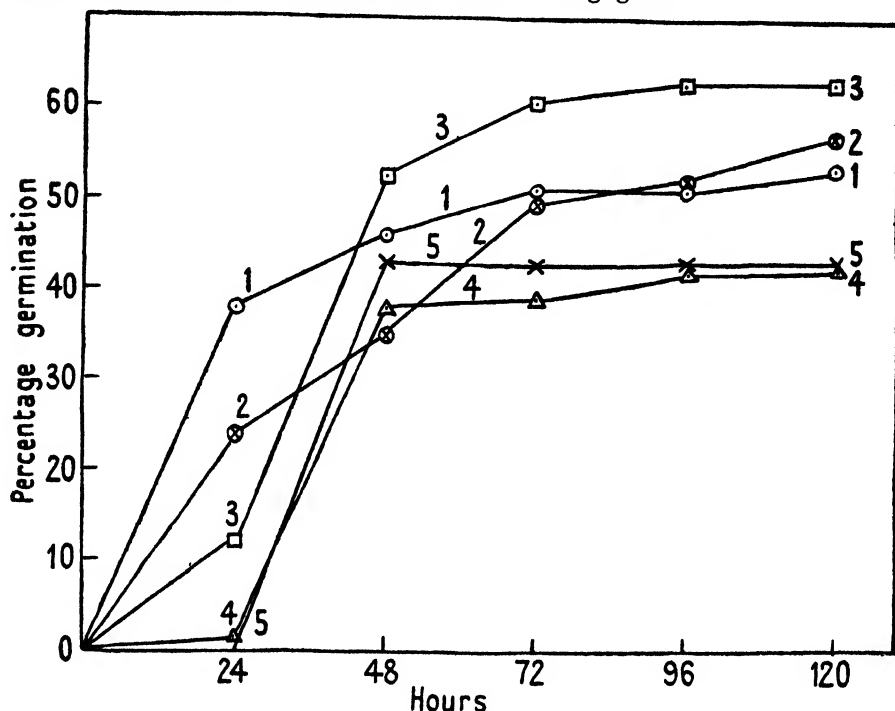


FIG. 7. Germination in successive zones along the vertical axis of cylinders in which composite cultures are established. The area over which measurements are made is divided into 5 superimposed zones, each 10 mm. broad. The zones are numbered serially, 1 being the highest and 5 the lowest in the vertical succession.

#### DISCUSSION OF RESULTS

The data of Fig. 4 suggest that at some stage in these composite cultures there is from the root along a lateral diffusion path a gradient of concentration of host material. To this must be attributed the decreasing relative frequency of germination with increasing distance from the host root. But the data of Fig. 5*b* and *c* also suggest that the period during which the stimulant is produced in the cultures is comparatively short. The germination frequency given by seeds in the immediate vicinity of the host root may represent a maximum germination capacity, but at remote distances from the root the lower frequency cannot be so explained. With a continuous production of the host material a prolonged exposure to a low concentration might be expected to give a germination frequency increasing slowly but continuously with time. Moreover in the conditions of culture the high temperature and moisture are such as to have the effect of pretreatment, and throughout the cultural period an increasing number of seeds are no doubt being brought to



the state at which germination occurs if the host stimulus is available. With continuous production of the stimulant this factor should also contribute to a percentage germination increasing with time. In fact percentage germination

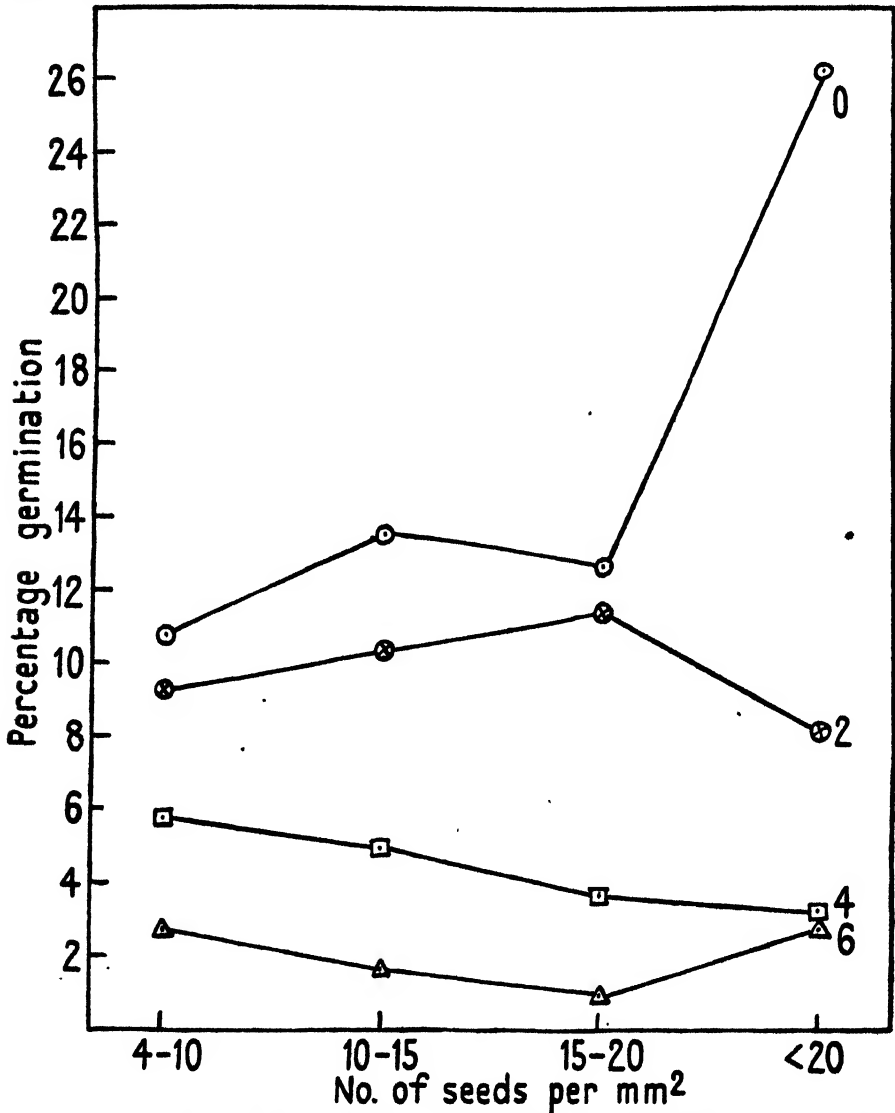


FIG. 8. The effect of density of sowing on germination at various distances (0, 2, 4, and 6 mm.) from a host root. Data are the means taken from seven replicated experiments, and represent final values obtained after culturing for 120 hours.

does not increase at any distance from the host root if laterals fail to be produced (as in the cultures of Fig. 5*b* and *c*) after 48 hours. This suggests that when the host root does not produce laterals the production of host factor in the cultures ceases after about the first 48 hours.

This change is undoubtedly related to a corresponding change that occurs in the state of the root system in the region of measurement at about 48 hours. The root begins its growth at the top of the cylinder, growing downwards between the glass and the filter-paper, and when it reaches the base of the column it continues to grow in the water in the Petri dish. The growth of the root from the top to the bottom of the cylinder occupies about 36–48 hours. Thus until the culture is 48 hours old the surface occupied by parasite seeds is at some point in contact with the root tip, but after that time it is in immediate contact only with relatively mature root tissue. The effect of the root tip on germination is shown by the data of Fig. 8. At the end of the first 24 hours the root has traversed about three-quarters of the length of the whole cylinder. At this stage a measurement of germination in the lowest zone cannot be made since the root tip has not reached this level. It is significant that at this stage the relative frequency of germination decreases from the top of the cylinder downwards. Corresponding changes in two conditions dependent on the growth of the root may also be noticed. At the end of the first 24 hours, successive zones from the top downwards have been occupied for progressively decreasing periods, and the time elapsed since the root tip traversed successive zones decreases from above downwards. The extent to which the changes in germination depend on each of these variable root conditions may be assessed from the subsequent changes in germination in each of the successive zones. If the high germination in the upper zones is due simply to the longer time during which they have been occupied by a length of root, then the frequency should continue to increase substantially in these zones after the 24-hour stage. This does not occur, and the course of the change in germination frequency in the two lowest zones after the 48-hour stage provides further evidence for the conclusion that the change in frequency with successive zones downwards at 24 hours is related to the length of time that has elapsed since they were successively traversed by the root tip. The root tip reaches the lower zones towards the end of the first 24 hours. In these the maximum germination occurs between 24 and 48 hours; after this stage the root tip leaves the cylinder and commences development in the Petri dish, and as this occurs further increase in the germination frequency in the lower zones ceases. Clearly, in the lower as in the upper zones the determining factor in the induction of germination is proximity to the tip and not to the general body of the root. This in turn suggests that the secretion of the host factor material is restricted to the tip of the root.

The reason for the cessation of the production of the host material in the cultures of Fig. 5*b* and *c* after 48 hours is now clear. During the first 48 hours the seed-occupied surface is being traversed by the only part of the root from which the host factor is secreted. This restricted region of the root, the tip, is carried away from the cylinder by growth just before or at about 48 hours, and consequently after this stage no further production of the stimulant occurs in the area occupied by the seeds.

Further evidence for the view that the active material is secreted only from

the tip is provided by the data showing the effect of lateral root development on germination frequency (Fig. 6). The development of laterals involves the production of secondary root-tips, from which the secondary stimulus to germination arises when the primary effect due to the passage of the main root has been exhausted.

The data of Fig. 7 indicate that the reaction of the seed to the host stimulus in the circumstances of these experiments is comparatively rapid. Thus in the upper zone of the cylinder 70 per cent. of the total germination observed at 5 days occurs within the first 24 hours, which suggests that the reaction to stimulation must be complete within that time. In this connexion the apparent slow rate of increasing germination frequency in the first 24 hours in the experiments of Fig. 4 requires some comment. These data represent the mean values for the whole length of the cylinder. At the end of 24 hours the position is no doubt similar to that of Fig. 7 at the corresponding stage, with a frequency which is high towards the upper end but decreasing rapidly towards the lower. Hence the low overall value which is dominated by low frequencies in lower and middle regions.

The conclusion suggested by the data of Fig. 7 that germination occurs within 24 hours after stimulation has been confirmed by the use of stimulating fluids separated from a host root. Serial observations with the hanging-drop technique on the same set of cultures have not shown any significant increase in percentage germination after the first 24 hours.

Other workers find that much longer periods are necessary for the completion of germination. Thus Saunders states that at 35° C. (the temperature used for stimulating germination in the present series of experiments) the first signs of germination are only apparent after 3 days. The difference is undoubtedly due to different experimental circumstances. In the present experiments the parasite seed is exposed to water at a comparatively high temperature before it is exposed to the host factor. In the experiments of Saunders and of other earlier workers the dry seed is subjected immediately to the influence of the stimulant. In these circumstances the moisture and high temperature after the application of the host factor are such as to induce the changes wrought by pretreatment, but since these changes only occur slowly they delay the operation of the host stimulus, and thus apparently prolong the time necessary for the seed to respond to this factor.

The observed effects of pretreatment indicate that the host factor only operates at a comparatively late stage in germination. It is evident that before the stimulating substance can have an effect certain developmental changes must occur in the seed. It is further evident from the data of Fig. 4 that these changes occur only comparatively slowly, whereas those induced by the host factor are extremely rapid.

The relation of the changes that occur in pretreatment to those that occur during the after-ripening of the seed are difficult to determine, particularly when the work is being conducted in England. Several observers (Kumar and Solomon, 1940; Pearson, 1912) have found that the seed of *Striga lutea*

will not germinate immediately after it is shed. A period of after-ripening must elapse before the seed can be induced to germinate. The sample from which the seeds for the experiments of this investigation were taken was at least 2 years old and therefore old enough to be in a fully mature state. Moreover, during the course of the investigation there has been no change in the reaction to pretreatment such as might occur with the development of after-ripening. Thus it would seem that pretreatment does not involve the acceleration of after-ripening but simply promotes changes that belong to the germination process as such.

The results of this investigation suggest an interesting possibility. Since the host stimulus is only effective at a late stage in germination it is possible by withholding the host factor to arrest the whole process without disturbing the metabolic systems of the tissues involved. A parasite seed which is dependent on a host for germination may therefore provide admirable material for the study of the complex process of germination.

#### SUMMARY

1. *Striga lutea* is an angiospermous parasite, the majority of whose seeds will germinate only when exposed to a stimulating material secreted by the roots of the host.

2. Two methods are described for studying the quantitative aspects of the host-parasite relation in germination.

3. Data are presented which show that the rate of germination after exposure to the host stimulus is enhanced by previous exposure of the seeds to moist, warm conditions. This is taken to indicate that the host factor only operates at a comparatively late stage in the germination process.

4. Although the majority of the seeds fail to produce a radicle unless exposed to the host factor, a small proportion with suitable treatment do so without host stimulation.

5. In composite cultures consisting of host roots and seeds of the parasite the frequency of germinated seeds tends to fall with increasing distance from the host root; this being the effect, it is suggested, of a corresponding gradient in the concentration of the material coming from the host root.

6. A comparison of the germination frequency along individual roots shows that roots from different seedlings vary considerably in the rate of secretion of the host factor.

7. Observations of germination frequency at intervals in successive zones of a vertical series across which a host root grows indicate that the secretion of the stimulant occurs only at the tip of the root.

8. Germination along a host root is enhanced by the production of laterals; this is attributed to the consequent development of additional root-tips.

9. It is suggested that, since with seeds of *Striga lutea*, and possibly with those of other similar parasites, germination can be arrested at a comparatively late stage by withholding the host factor without at the same time disturbing

normal metabolic processes, such seeds may provide favourable material for the study of germination.

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# **The Scope, Technique, and Interpretation of the Results of Experiments on Absorption of Salts by Storage Tissue.**

## **A Reply to Criticism**

BY

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AND

A. D. SKELDING

SOME five years ago we began a fresh attack on the problems of salt absorption by plant cells, and in 1940 we published some of the early data obtained in this work. These were contained in two papers (Stiles and Skelding, 1940 and 1940 *a*) dealing with the course of absorption by carrot root tissue of a number of potassium and manganese salts in a range of concentrations. A recent article in this journal by Steward and Berry (1943) containing an attack on the first of these papers gives so unreal an impression of our outlook and work, and contains so many inaccurate and misleading statements, that a correction of at least the more extreme of these appears necessary. The article implies, or so it seems to us, that we have an antiquated outlook, an indifference to the work of the last quarter-century, use inadequate methods, and interpret incorrectly our experimental results. We are also said to have criticized Steward and his collaborators on a number of points, of course without justification.

It appears to us that the major criticisms of Steward and Berry have been induced by (1) their failure to appreciate the intended scope of the work we had projected, and the place of the work we published in 1940 in the whole scheme, (2) a wholly false assumption concerning the degree of agreement between replicate experiments in our work, and (3) a propensity for discovering adverse criticism of their own work where none was intended. Our reasons for coming to this conclusion will appear in the course of this note.

Since the criticisms of Steward and Berry mainly concern the scope of our work, the technique we employed, and the interpretation of the results obtained, we will discuss these matters in that order.

*Scope of the work.* We thought we had made it clear that we intended to obtain data on the absorption of a wide range of salts by a variety of different kinds of cells, and that in the first place we were confining our attention to storage tissues. We pointed out that with these were available data with only a few salts, and that the tissues used and concentrations employed varied from experiment to experiment. We summarized the present position of our knowledge on the course of absorption of salts by storage tissues in which

actual determination of the ions concerned had been made in our Table 1<sup>1</sup> in order to emphasize the fact that existing data of the kind we were proposing to get were relatively few.<sup>2</sup> As a necessary preliminary to a more detailed and intensive analysis of absorption of individual salts by various tissues, it therefore appeared to us very desirable that we should have a systematic survey of the course of absorption of a wide range of salts presented to the tissues in a range of concentrations. Our first two papers gave results of the first instalments of this survey. Nothing more is claimed for this work than that it is the first part of this preliminary survey. The more detailed analysis we hope is to follow.

Our immediate aim being solely the simple one of obtaining a much greater body of data with regard to the absorption of salts by storage tissues, we were not at the moment concerned with a review of salt absorption in general, and for that reason did not discuss the work of Osterhout, Hoagland, Lundegårdh, and others which has contributed much to the development of our knowledge and views on the salt relations of plant cells. The suggestion that we were ignorant, or at least inappreciative, of the contributions of these and other distinguished plant physiologists we find particularly objectionable. It should be sufficient to point out that one of us has, in the course of twenty years between the two wars, repeatedly called attention in the pages of 'Science Progress' to much of the work we are now charged with neglecting, while in his Presidential Address to the Botanical Section of the British Association at Cambridge in 1938 he was at some pains to indicate the great change in outlook on problems of salt absorption which had resulted from the work of the 1919-38 period, the work which we are now charged with treating 'almost as though this fertile period had never been'. The suggestion that we do not appreciate the vital nature of the experimental material is equally unjustified.<sup>3</sup>

*Experimental technique.* The experimental technique used for obtaining data of salt absorption by storage tissue was the same in principle in the experiments of Steward and Berry and in ours. It consists in immersing a number of discs of tissue in a volume of solution under certain conditions which are kept as constant as possible and estimating intake of salt ions by

<sup>1</sup> Steward and Berry (1943, p. 233) appear to object to an entry in this table concerning an experiment they made on the absorption of potassium bromide by artichoke. We stated that they examined anion absorption only, thus indicating that the absorption of potassium was not actually measured. They now write 'Stiles and Skelding say that the bromide ion *alone* was observed'. We are aware that from a consideration of conductivity data they drew certain conclusions with regard to the absorption of potassium, but after a re-reading of their paper we are still unable to find a single actual determination of potassium recorded therein. The conditions of this experiment were unusual, and to indicate this briefly in a footnote to the table we noted 'Solutions changed daily'. Admittedly the conditions were more complex than this, but as it takes Steward and Berry several lines of print to indicate what they were (l.c., p. 233), we may be pardoned for the brevity of our description, even if it involved an inexactitude.

<sup>2</sup> Steward and Berry misrepresent us by saying that 'we make it appear that quantitative experiments are still a *novelty* in this field' (*italics* ours).

<sup>3</sup> 'One significant fact does, at least, emerge from the information so far acquired, namely, the absorption of dissolved substances by plant cells is as much a vital process as the respiratory function' (Stiles, 1938).

the tissue. It is a development of a procedure introduced nearly thirty years ago by Stiles and Jørgensen, and it would indeed be strange if in the course of that time the procedure should not have undergone modification and improvement. No doubt a most important improvement is that resulting from the recognition of the importance of oxygen as a factor in salt intake, and credit is due to Steward and Berry for emphasizing this fact.<sup>1</sup> The chief differences in actual technique used by Steward and Berry and by us lay in the size of the containers, the absolute and relative quantities of salt solution used, the method of maintaining oxygen supply, and the method of measuring the amount of salt absorbed. Steward and Berry used large containers of 4 litres capacity (not 2 litres as we stated earlier), with 40 to 45 gm. of tissue in two litres of solution which was continuously stirred and aerated. We used containers of 420 or 600 ml. capacity with 200 ml. of solution and about 20 gm. of tissue, while the oxygen supply to the discs was maintained by keeping the containers gently shaken and open to the air through a hole, 1 cm. in diameter, in the stopper of the bottle.

In discussing this arrangement we endeavoured to show that it was adequate for our purpose of supplying the tissue with oxygen, and we compared it with the arrangement of Steward and Berry in this regard. In doing this we were not criticizing the arrangement of Steward and Berry, and it is surprising to find our remarks, aiming at no more than an evaluation of the adequacy of our own arrangement, so interpreted. A main reason for not adopting the system of Steward and Berry is its bulk. If an arrangement involving equipment only one-tenth the size gives results of a sufficient degree of accuracy, there is much to be said for it, for reduction in size generally means that more experiments can be done in the same time. Space and time are not considerations to be lightly disregarded. We would indeed emphasize this point. It is our very strong opinion, which we know is shared by other physiologists and biochemists, and which we should have thought was obvious to all with experience in these fields, that the development of methods which will permit the winning of experimental data at a much more rapid rate than has hitherto been possible, is essential if the solution of many of the problems confronting us is to be achieved within a reasonable time. Also we hold it of equal importance to develop the methods, and make use of the instruments, which have been developed in recent years for the measurement of small quantities. We note that Steward and Berry prefer 'standard chemical methods' to the use of the polarograph.<sup>2</sup> It is true that without special arrangements the polarograph does not separate potassium from sodium, but we pointed out this fact and explained why we did not consider this limitation of the method introduced any significant error in the results, while we considered any disadvantage resulting from this limitation was

<sup>1</sup> It is, however, only fair to point out that the importance of a supply of oxygen to tissue in experiments of this kind was very clearly pointed out by Stiles in 1927.

<sup>2</sup> 'Stiles and Skelding now favour the use of the polarograph' (Steward and Berry, 1943, p. 226). Why *now*, and why only the polarograph? We mentioned five different methods available for measuring small quantities and actually made use of three of them.



outweighed by two advantages, one the ease and rapidity of the determinations, the other the sensitivity of the method which enables quantities of the order of a microgram or less to be determined. We are not aware of any 'standard chemical method' for determining potassium which has a sensitivity remotely approaching this. That potassium offers particular difficulty in its estimation in biological material is well recognized.

As regards the method of aerating the culture solutions, Steward and Berry (1943, p. 222) say that we 'criticize both the flowing air technique and the conclusions which have been derived by its use'. We made no such criticisms, and on re-reading our remarks on the subject we are at a complete loss to know why Steward and Berry should have made such a statement. It is a complete misrepresentation of our opinions.<sup>1</sup> Steward and Berry, however, now attempt (l.c., p. 223) to throw doubt on the adequacy of the aeration system we employed. Actually we showed that with our arrangement the absorption of bromide from rubidium bromide of concentration 0.002 N was of the same order as that obtained by Steward and Harrison with their arrangement. The comment of Steward and Berry on this is that 'a critical test demands that random-sampled batches of discs from the same stock should be submitted in concurrent experiments to the exact conditions of Steward and Harrison and of Stiles and Skelding'. To this we have to say that in the first place such an exact comparison was neither aimed at nor necessary, while in the second place we made experiments which are on record (1940 a) in which the absorption of 'random-sampled batches of discs from the same stock' were 'submitted in concurrent experiments' to our normal technique as regards aeration, and to aeration with a continuous current of purified air (carried in from outside the building). Admittedly certain conditions in these experiments were different from earlier ones, but for at least four days no very great difference occurred in the absorption under the two methods of aeration.

Steward and Berry make great play with the divergence between the absorption of bromide from rubidium bromide in three separate experiments we made. Thus after absorption for 94 hours the relative amounts of absorption in the three experiments were 44, 51, and 37. The differences were in all probability related to the different previous histories of the tissue used in the three experiments and certainly not to differences of aeration as Steward and Berry suggest. But the point is that the sole object of these experiments was to discover whether the simple aeration technique brought about a marked suppression of ion uptake as compared with a technique involving aeration

<sup>1</sup> The use of a continuous current of air in work with plant cultures was, as far as we are aware, first used by Hall, Brenchley, and Underwood in 1913, when they showed the great effect it had in increasing the rate of growth of barley in water culture. The method was used by Stiles and Jørgensen in 1917, when the same favourable effect of a continuous current of air was found with barley and balsam. With this earlier experience of one of us, why should we 'criticize the flowing air technique?' Ten years later, but still five years before the first of the papers of Steward's series appeared, one of us had written with regard to the technique of experiments with discs of storage tissue: 'probably aeration would be a more satisfactory method of maintaining the healthy condition of the tissue' (Stiles, 1927).

with a continuous current of air. The data we obtained showed that it did not, and the experiments thus fulfilled their purpose. We were not obtaining critical data on the absorption of rubidium bromide nor were we concerned with an exact replication of conditions. In assuming that in our work on the absorption of salts by carrot tissue the agreement between replicates was no better, or maybe worse (Steward and Berry, l.c., p. 229), than in the trials with rubidium bromide, Steward and Berry made an erroneous and gratuitous assumption for which in 1943 there was no justification, since we had published long before (Stiles and Skelding, 1940 *a*) data showing the absorption of manganese chloride in duplicate cultures. Since so much has been made of this point by Steward and Berry we may be allowed to quote in the Table the results of one experiment with manganese chloride in which the tissue

TABLE  
*Absorption from Manganese Chloride by Duplicate Samples of Carrot Root*

| Daily period. | Manganese. |            | Chloride.  |            |
|---------------|------------|------------|------------|------------|
|               | Culture 1. | Culture 2. | Culture 1. | Culture 2. |
| 1             | 0·72       | 0·73       | 0·45       | 0·42       |
| 2             | 0·57       | 0·57       | 0·44       | 0·43       |
| 3             | 0·42       | 0·42       | 0·40       | 0·34       |
| 4             | 0·32       | 0·365      | 0·31       | 0·32       |

was exposed to a fresh solution (0·001 M) each day and the absorption of each ion during successive 24-hour periods measured. These values are very representative of the degree of agreement between replicates which we obtained throughout our work. In fact determinations of ionic concentrations in replicate cultures rarely differed by more than a few per cent. and agreement was often much closer. When Steward and Berry (l.c., pp. 224-5) tell us that in their work 'it has been the common practice to compile data in a single experiment from as many as eight, or even twelve to fourteen, separate cultures' we may be pardoned for wondering if their replication was any better than ours. In any case, the remarks of Steward and Berry on the lack of control of conditions in our arrangement are based on a completely erroneous assumption and are consequently valueless.

As regards the method of measuring ion uptake we have made analyses of the external solution while Steward and Berry express a preference for analysis of the tissue. We have set forth our views on the relative merits of these two ways of following ion uptake and to these we adhere. We are not without experience in these matters, for in the course of the last few years many hundreds of determinations of elements in plant tissues have been made in our laboratory. But to avoid further misunderstanding we might add, what should appear self-evident, that under certain conditions of experiment either one or other of these methods might be the only one possible and the question of choice would not arise. To state, as Steward and Berry do, that 'we seem to deprecate the value of analysis of the tissue', is not a correct representation of our views.

*The interpretation of the results of experiments.* It is, of course, quite legitimate for Steward and Berry to put their own interpretation on our time-absorption curves, and they should therefore not object if we do the same with theirs. But we do not agree with them that the phasic character of the curves we obtained is due to casual fluctuations, or that we should not have observed it had we not approached the interpretation of the curves 'from the point of view of Stiles and Kidd'. As a matter of fact, when this phasic character of the curves was first observed by us we thought, like Steward and Berry, that it was probably to be ascribed to fluctuations of this kind, though we could find no reason why such fluctuations should occur, having regard to the close control of the conditions of our experiments. But when this phasic course constantly recurred in all replicates with a number of different salts, we were forced to conclude that it had some real significance. Indeed, work on the meaning of the temporary slowing-down of absorption towards the end of the first period was actually proceeding, and what appeared to be some explanation of it coming to light, when work on the problem had to be suspended. In so far as Steward and Berry were influenced in their remarks on the course of our curves by their false assumption regarding control of conditions their criticisms are meaningless.

Steward and Berry suggest that we have not dealt justly with the results published by Steward and Harrison on the course of absorption of rubidium bromide by potato. We should certainly have said that in their experiment the uptake of rubidium unaccompanied by bromide occurred for about a day,<sup>1</sup> instead of a day or two, but whatever the respective merits of displaying results by continuous curves (our method) or by straight lines of nearest fit (the method of Steward and Harrison), we are of the opinion that our curves more exactly represent the primary data obtained experimentally by Steward and Harrison than do the graphs in their Fig. 4, in which the line  $[Br] = 0.173(t-9.0)$  only passes through two of the five experimentally determined points. If this equation truly represents the course of bromide intake then the errors (+deviations) in determining three of the five bromide concentrations on which the relation is based were 68, 9, and 6 per cent. respectively, and with errors of this magnitude in three out of five values on which the supposed relation is based we hold that the evidence is not adequate to decide whether the absorption/time relation is linear or not. We are not denying that it may be, but merely stating that more data are required to decide the point.

In commenting on our remarks on the effect of temperature on salt absorption, in which we pointed out that temperature coefficients could not be calculated from the data Steward presented earlier, Steward and Berry now say: 'Doubtless Stiles and Skelding had in mind that in the work of Stiles and Jørgensen (1915) to which Steward (I, 1932) referred, data were given on the course of the process measured with time. Stiles and Jørgensen, however, gave no proof that they really measured *absorption* of H ions'. Now what we had in mind was the correct way of calculating temperature

<sup>1</sup> A small intake of bromide was observed after 21.7 hours.

coefficients and not the work of Stiles and Jørgensen; and what they measured in 1915 is completely irrelevant to the argument. Nor did we call in question the sigmoid character of the absorption/temperature curve as Steward and Berry (l.c., p. 235) now assert. We merely called attention to a way in which temperature coefficients could not be calculated.

It does not seem to us worth while to deal with a number of trivial points raised by Steward and Berry. Where these are relevant we may have occasion to deal with them in later papers of our series. For the rest we only ask that those interested should consult our original papers and form their own judgement. But one last question can scarcely be passed over since it has been the subject of constant misrepresentation. That is the adsorption hypothesis. On this Steward and Berry (1943, p. 226) write: 'The analysis of tissue fluids was necessary to ascertain the state of the absorbed salt in the cells, a necessity partly due to the adsorption hypothesis *for which Stiles was originally responsible* (italics ours)'. Now Stiles was not, and has never claimed to be, 'originally responsible' for the adsorption hypothesis. It is true that he has always held that adsorption cannot be disregarded as a possible factor in salt absorption, and in 1927 he suggested tentatively, in order to bring into harmony the results of experiments on permeability with the plasmolytic method and the facts then established about the entry of ions against the concentration gradient, that salts might both be adsorbed by the protoplasm and diffuse through the protoplasm into the vacuole. That was seventeen years ago, and he would certainly modify views then expressed in the light of more recently acquired knowledge. But so far from the adsorption hypothesis being due to him, he has urged, first that there was not sufficient evidence to justify its acceptance, and later that adsorption could not be the only factor in salt absorption. The accuracy of the statement of Steward and Berry may be judged from the following quotations from the writings of Stiles over a period of twenty-one years:

'The data presented are inadequate in themselves to justify the conclusion that absorption of salts by the cell is an adsorption process, and no proposals are put forward as to the mechanism of salt intake by the cell' (Stiles and Kidd, 1919).

'Agrément with the adsorption equation cannot be regarded as sufficient evidence in itself' (Stiles, 1924).

'This equation is that representing the relationship between concentration and amount of adsorption, but, as repeatedly pointed out by the writer, it does not prove that the absorption of the salts is a process of adsorption' (Stiles, 1936).

'Adsorption cannot be the principal factor controlling this absorption' (Stiles and Skelding, 1940 a).

A few words in conclusion. It is, of course, perfectly clear that at the moment our approach to the problems of salt absorption is different from that of Steward and his collaborators. The difference does not lie, as Steward and Berry imply, in our lack of appreciation of the vital character of salt absorption. Rather it lies in this, that Steward and his collaborators prefer now to attempt an analysis in detail of data provided by experiments with one

or two salts, while we feel that as a preliminary to such intensive study a broad systematic survey of absorption by a representative range of salts is desirable, as otherwise generalizations may be drawn from specific cases and later found to have no general validity. In saying this, we are far from deprecating the value of such detailed analysis, but results, both published and unpublished, which we have so far obtained in our general survey, confirm us in our belief that our caution against drawing conclusions from too scanty data is justified. But that our approach to the problems of salt absorption is different from that of Steward and Berry seems to us an inadequate reason for their attributing to us an outlook we repudiate, views we do not hold, and criticisms we did not make.

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# Cladophorella calcicola nov. gen. et sp., a Terrestrial Member of the Cladophorales

BY

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With four Figures in the Text

THE alga that forms the subject of the present communication has been found in some quantity on oolitic limestone rocks in certain of the damper hothouses (tropical fern house, stove, palm house) in the Cambridge Botanical Gardens. The rock-fragments in question, in part certainly remains of some building, are either scattered under the stages or placed round the soil in which some of the larger plants are rooted. A similar rock has been employed in the construction of parts of the rock-garden. It is a coarse even-grained, non-shelly oolitic limestone, comparable with that extensively used in Cambridge college buildings. It especially resembles the Ketton freestone of Rutland.<sup>1</sup> I have been unable to discover the alga of the hothouses on the numerous other similar substrata found outside, so that the possibility that it may be an introduced tropical form, which alone finds conditions favourable to its development in the warm humid atmosphere of the hothouses, must be envisaged. Its apparent restriction to the oolitic rock-fragments, despite the fact that other kinds of substrata are available, indicates that it is a pronounced calcicole. It is, moreover, probable that it is a shade form, since it occurs more abundantly on the rocks under the stages and on them more particularly along the sides.

An undoubted member of the Cladophorales, it constitutes so far as I am aware the first truly terrestrial member to be described among Cladophoraceae and, apart from the genus *Wittrockiella* of doubtful affinity, the first example in the order. Its distinctive morphology warrants the establishment of a separate genus, for which I propose the name *Cladophorella*. It exhibits pronounced adaptations to a terrestrial existence, not least in the presence of what appears to approximate to a true cuticle on the upper parts of the erect-growing threads.

## THE VEGETATIVE SYSTEM

*Cladophorella* occurs as short dense tufts, approximately 2–5 mm. high and of a bright green colour; when thoroughly moistened the colour is deeper. The threads composing the erect tufts all reach to about the same height and

<sup>1</sup> I am indebted to Dr. L. Hawkes of Bedford College, University of London, for this information.

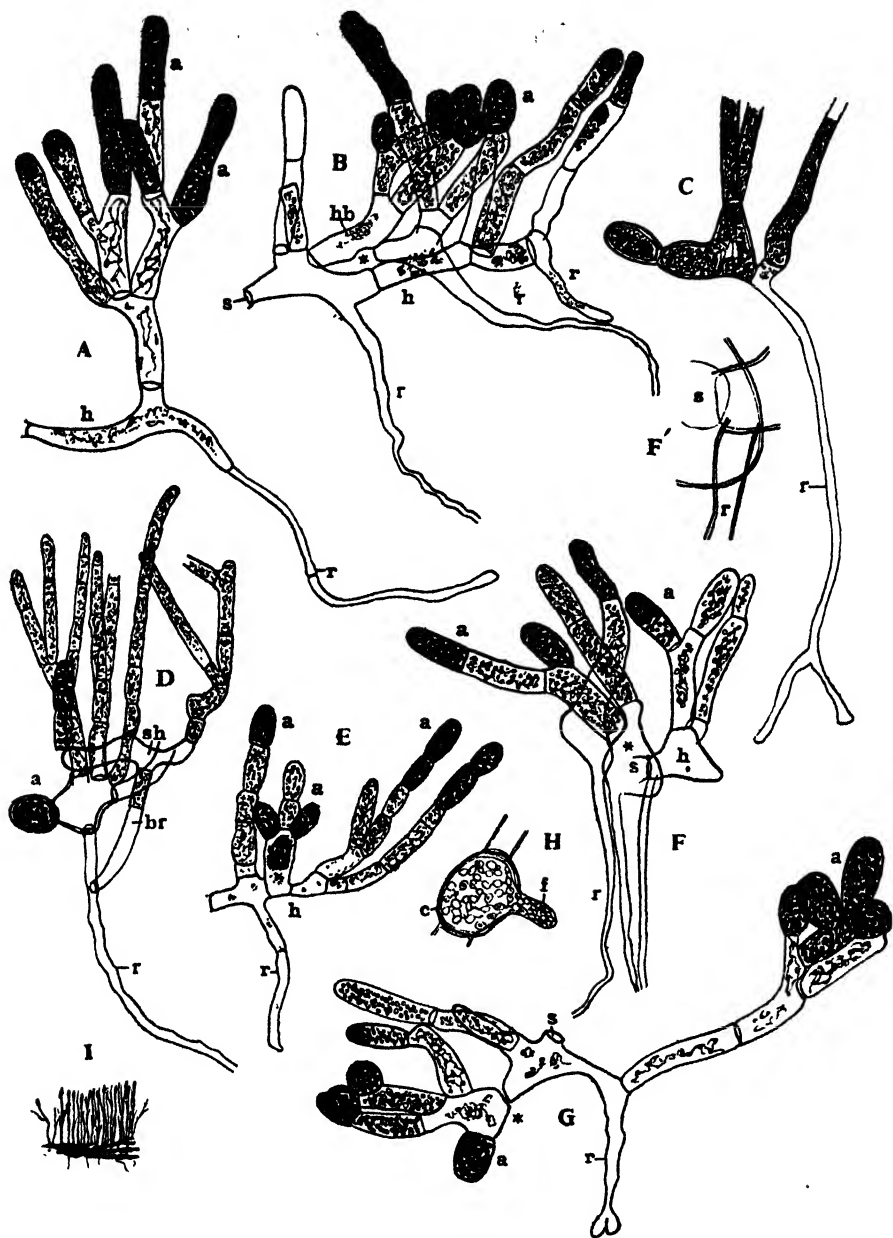
are often markedly inclined in one direction, suggesting a tropic response; it seems probable that this may be of the nature of negative geotropism, since there is no obvious relation to the direction of the light. The height attained probably depends on the amount of available moisture. Material which has been exposed to drought may show a white efflorescence of carbonate of lime (readily soluble in dilute hydrochloric acid) on the erect-growing threads.

The typical habit of the alga is shown in Figs. 1 B, 1 E, 2 A, and 2 K. The erect threads arise from a horizontal system (*h*) which is composed either of more or less cylindrical cells, in general 3–5 times as long as broad (Figs. 1 B, 2 A), or of shorter cells of irregular shape (Figs. 1 F, 2 K); the latter are commonly almost isodiametric, sometimes even broader than long, and may be produced on their upper side into two short arms terminating in erect branches (cf. Fig. 2 K). The cylindrical cells vary between 36 and 51  $\mu$  in width, the others may be as much as 100  $\mu$  in diameter. Each cell of a horizontal thread usually bears one or more branches, while certain of them are produced downwards into rhizoids (*r*) which are often of considerable length, although sometimes short (Fig. 1 E), usually devoid of septa, and only exceptionally branched.

The horizontal threads produce branches that grow out horizontally (Fig. 1 B, *hb*), as well as those which grow erect. The horizontal branches extend between the erect threads so as to form a complex web which is not easily disentangled (cf. Fig. 1 I). It is not uncommon to find as many as three sets of horizontal threads situated the one above the other. When such a web is teased apart, there is a great tendency for the horizontal branches to break away from one another at the septa, and most of the branch-systems shown in Fig. 1 are probably fragments of more extensive ones. A detached horizontal branch appears in Fig. 1 B, its point of insertion on the parent cell being indicated by an asterisk. It is highly probable that such fragmentation also occurs in nature and that contiguous tufts of erect branches arise from several independent horizontal systems, some or all of which may have originated from one another. I do not find it possible to estimate the extent of any single plant, because of the threading of the horizontal branches between the bases of the erect filaments and the readiness with which they break away from one another.

The tendency towards rupture at the septa is not, however, confined to the points of origin of horizontal branches. It also ensues readily between the individual cells of the horizontal threads (cf. Fig. 1 G\*) and especially between those which are almost isodiametric and of irregular shape. In Fig. 1 F, for instance, the right-hand cell of the three horizontal ones has become detached from the others and the scar (*s*) left on the wall of the middle cell is plainly visible; the point of rupture is shown on a larger scale in the figure above (Fig. 1 F'). Examination of teased-out material always discloses individual specimens such as are shown in Figs. 2 B and 2 I, which almost certainly represent isolated portions of horizontal threads with their rhizoids.

It is not altogether clear what determines the production of rhizoids. Sometimes they originate from a considerable number of the cells of the



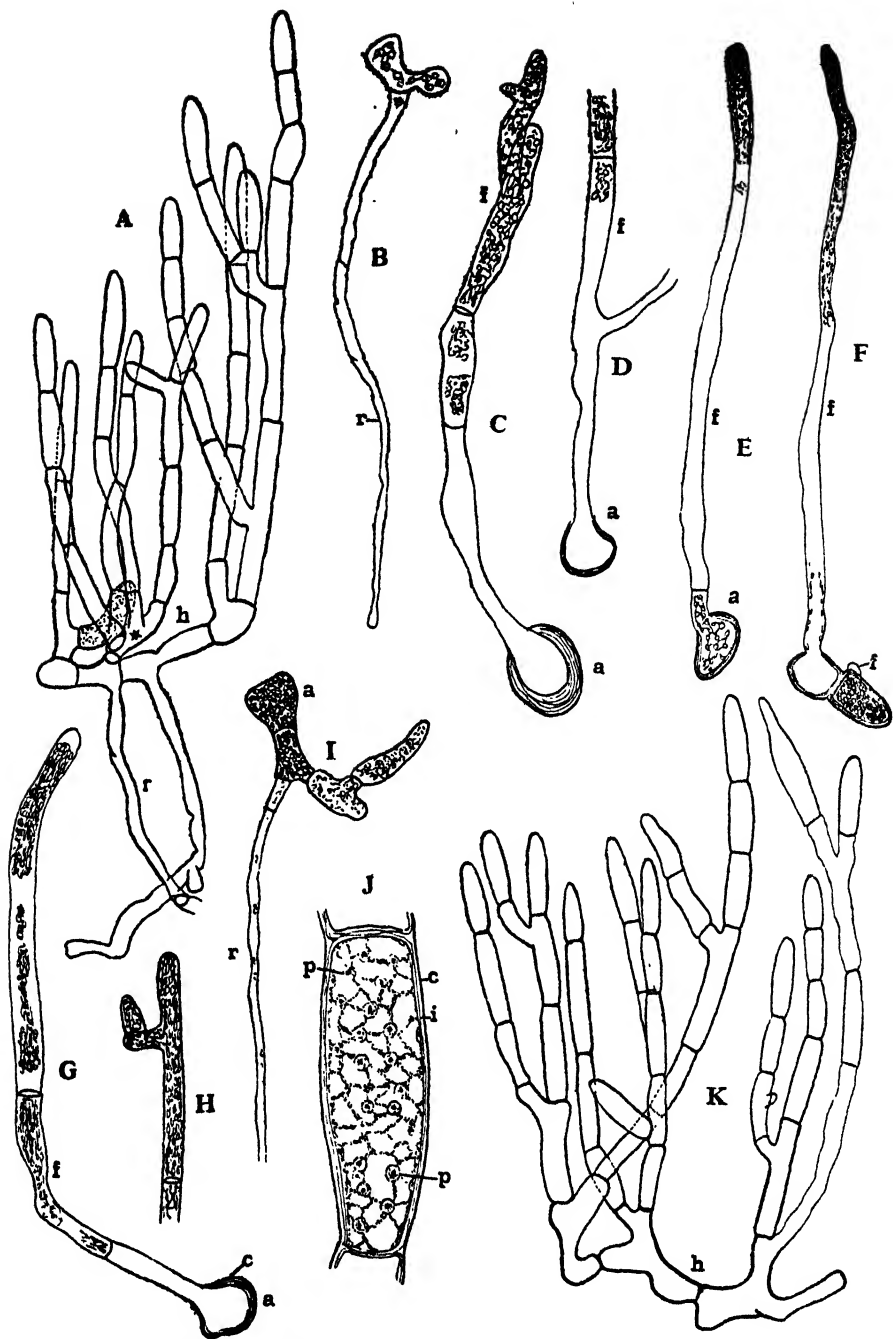
FIGS. 1A-1I. *Cladophorella calcicola* n. gen. et sp. A-G, portions of various plants, in A and C with terminal, in D and G with median rhizoids; B shows branching of the horizontal system and at \* a detached branch; D, formation of a secondary horizontal system (sh) from a branch (br) of the rhizoid; F, a horizontal thread composed of almost isodiametric cells in which the right-hand one has broken away from the others; F', the region of detachment of this cell on a larger scale. H, early stage of germination of an intercalary akinete. I, diagrammatic representation of a complex tuft, as it appears when mounted under the microscope. \*, places at which rupture has occurred; a, akinetes; br, branch of rhizoid; c, cuticle; f, germinal tube; h, horizontal threads; hb, branch of same; r, rhizoids; s, scars left after detachment of cells or branches; sh, secondary horizontal system. (A-C, F, G  $\times 95$ ; D, E  $\times 75$ ; F'  $\times 230$ ; H  $\times 170$ ; I  $\times$  about 2.)



horizontal threads (Figs. 1 B, 1 F, and 2 A), but in other instances there are no such rhizoids (Fig. 2 K). I suspect that rhizoid-formation takes place only from cells of horizontal threads that are in contact with the substratum, and that specimens like that shown in Fig. 2 K represent detached horizontal branches which extended above the level of the latter. It is quite exceptional for more than one rhizoid to be formed by a cell. The rhizoids are for the most part appreciably narrower (diam. 12–14  $\mu$ ) than their parent cells, although sometimes relatively wide (diam. up to 20  $\mu$ ) near their point of origin. They usually have only remnants of cell-contents and are generally not cut off from the parent cell by a septum (Figs. 1 B, 1 C, 1 E, and 2 A). They are commonly irregularly undulate over part of their length (Fig. 1 B), sometimes with local inflations, and the relatively thin membrane often shows slight folds (Figs. 2 A, 2 B). Although usually devoid of septa (Figs. 1 B, 1 C, 1 F, and 2 A), a few are sometimes present (Figs. 1 A and 2 B). The distal end of intact rhizoids is generally somewhat expanded, sometimes even lobed (Fig. 1 G), and is loosely adherent to small particles in the substratum. Occasionally the rhizoids develop a few short branches (Fig. 2 A), but longer branches (Fig. 1 C) are exceptional. An unusual condition is shown in Fig. 1 D; here the rhizoid has produced an upgrowing branch (*br*) a little below its point of origin, and this branch has given rise to a secondary horizontal system (*sh*) bearing a number of erect threads. I have also seen stages which suggest that rhizoids may occasionally pass over into a new horizontal system at their distal extremity.

The rhizoids usually originate from the underside or from the flanks of the cells of the horizontal threads, often near one end (Figs. 1 B, 1 E, 1 F, and 2 A), but it is not uncommon to find instances in which the rhizoid appears as a direct continuation of a horizontal thread (Figs. 1 A, 1 C). Another condition, with a more or less median rhizoid, is shown in Fig. 1 G. This is quite frequent, the median rhizoid often arising from a horizontally extended cell bearing branch-systems on both of its arms. These diversities are responsible for the very varied appearance of the detached fragments observed in teased-out material. I suspect that, like the diverse form of the cells of the horizontal threads, they depend in part at least on the location of the plants in the variously shaped crevices of the surface of the oolitic rock.

In an older plant which has produced erect branches from the horizontal threads the cells of the latter usually possess only scanty contents (Figs. 1 B, 1 G) or are altogether empty (Fig. 1 F). Growth of the threads probably takes place almost entirely by elongation of the end-cells, and it is evident that, as growth proceeds, the bulk of the contents concentrate in the upper cells of the erect threads. Filaments which are actively growing commonly show a marked accumulation of the cell-contents near the tip (Figs. 2 E and 2 F). The degree of development of the erect threads probably depends on the density of their juxtaposition as well as on surrounding conditions. The former factor perhaps determines the extent of branching, the latter almost certainly the height attained. When the erect threads are not densely crowded they



FIGS. 2 A-2 G. *Cladophorella calcicola* n. gen. et sp. A, K, parts of plants with well-developed erect threads (cell-contents omitted); at \* in A a detached vertical branch. B, I, detached fragments of horizontal systems. C-G, germination-stages of akinetes. H, tip of a germinal tube, with a horizontal branch. J, vegetative cell from an erect thread. a, akinetes; c, cuticle; f, germinal tube; h, horizontal threads; i, inner (pectic) layer of membrane; p, pyrenoids; r, rhizoids. (A, B, D-I, K  $\times 90$ ; C  $\times 180$ ; J  $\times 360$ .)

are usually well branched (Figs. 1 A, 2 K); on the other hand, when closely aggregated they show little or no branching (Figs. 1 D, 2 A). Exposure to drought terminates the growth of the erect threads and the cells at the tips of the branches are converted into akinetes (1 A, 1 B, 1 E, 1 G, a). When there is sufficient moisture growth continues longer, and the erect threads are composed of more numerous cells and attain a greater height (Figs. 2 A, 2 K).

The erect threads are in general narrower than the horizontal ones. They are usually 24–36  $\mu$  wide (rarely up to 46  $\mu$ ) and 3–7 times as long as broad, although occasional longer cells are found. Branch-formation takes place as a general rule from the upper part of the parent cell, sometimes immediately beneath a septum (Figs. 1 A on the right and 1 E and 1 G), but quite frequently some way below it (Figs. 1 A on the left, 1 F, and 2 K). In most instances, but not invariably, the first septum within the branch arises at some little distance above its base so that the parent cell is branched near its summit. The branches remain as lateral outgrowths and eversion, if it occurs at all (cf. Fig. 1 B), is rare. Branching takes place in all planes and, in the horizontal threads, the branches not uncommonly arise from the flanks of the cells. Sometimes in such threads branches arise from the two sides of a single cell (Fig. 1 F), but such branches mostly originate at different places along the length of the cell and are rarely opposite. The formation of two branches by a cell of the erect threads seems to be exceptional (Fig. 1 E).

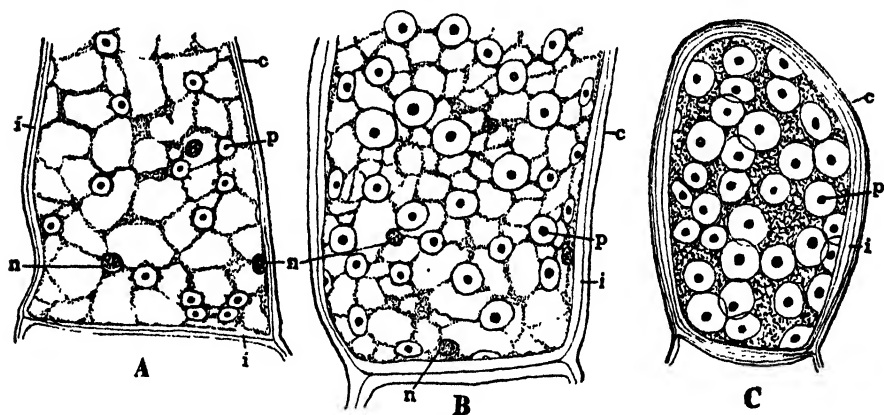
#### CELL-STRUCTURE

The cell-structure is typically cladophoraceous, that is to say, there is a parietal, reticulate chloroplast containing a considerable number of pyrenoids (Fig. 2 J) and, internal to it, a relatively small number of nuclei. The details of cell-structure were studied on fresh material and on material fixed in Belling's modification of Navaschin's fluid, with subsequent staining in lactophenol cotton blue or iron alum haematoxylin.

In the ordinary vegetative cells (Fig. 3 A) the chloroplast forms an irregular network, with wide meshes of unequal size and shape separated by relatively narrow strands and with rather frequent pyrenoids (*p*) lodged in the angles of the network; certain wider areas of the chloroplast lack pyrenoids. In the lower cells of older plants the chloroplast is commonly more or less disintegrated, consisting of a number of separate pieces, some containing pyrenoids, and in part linked to one another by delicate strands of chloroplast-substance. This condition of the contents is quite the rule in many of the cells of the horizontal threads after they have produced several-celled erect filaments, and in plants forming akinetes may be met with in most of the lower cells of the erect branches.

In the end-cells of branches which are about to become converted into akinetes, and often also in one or more of the underlying cells, there is a great increase in the size and in the number of the pyrenoids (Fig. 3 B, *p*); at the same time the reticulum of the chloroplast becomes denser and more complex,

with smaller intervening meshes. I think it probable that multiplication of pyrenoids is effected solely by division; they are often found in pairs, as in the lower part of Fig. 3 A. Ultimately, as the cell reaches the condition of a mature akinete, the chloroplast becomes very dense, harbouring very large numbers of closely crowded pyrenoids (Fig. 3 C, *p*). There is in such cells



FIGS. 3 A-3 C. *Cladophorella calcicola* n. gen. et sp. Cell-structure; material fixed in Belling's modification of Navaschin's fluid and stained in cotton blue in lactophenol; all drawings in surface view. A, part of a vegetative cell; B, part of a subterminal cell; C, akinete. *c*, cuticle; *i*, inner (pectic) layers of membrane; *n*, nuclei; *p*, pyrenoids. (A, B  $\times 860$ ; C  $\times 650$ .)

often so much granular material (probably largely starch) between the pyrenoids that the chloroplast-substance is difficult to detect, although in particularly favourable instances (Fig. 4 G) occasional connecting strands are recognizable. It seems not improbable, however, that in mature akinetes the chloroplast consists largely of separate pieces, each occupied by a large pyrenoid.

In the end-cells of actively growing erect threads the chloroplast often presents a rather different aspect, the reticulum in the upper (Fig. 4 F) or in the greater part of the cell being markedly drawn out in the longitudinal direction so that its strands run more or less parallel to one another, with narrow intervening meshes. The appearance is then more like that of the chloroplast of an *Oedogonium* than of a *Cladophora*. At the actual tip, however, the reticulum is more polygonal in character and usually very dense, as though it were piled up against the growing summit.

The nuclei rarely showed good fixation. They are probably spherical or slightly oblong and fairly evenly spread (Figs. 3 A, 3 B, *n*). They are not numerous, about 8-12 in the ordinary vegetative cells, probably rather more numerous (up to 18) in the akinetes. Since the latter are on the average appreciably shorter than the vegetative cells, this implies a probable doubling of the number of nuclei. In a few instances a single nucleolus was recognizable.

The membrane of the vegetative cells is for the most part relatively thin ( $1.7-2 \mu$ ). A surface pellicle (Figs. 3 A, 3 B), appearing in optical section as

a thin dark line, is readily distinguished from a much less highly refractive inner layer (*i*) which is colourless and presents a whitish appearance (cf. Brand, 1901, p. 483; 1908, p. 121). The inner layer consists of pectic compounds (Brand, 1901, p. 487), since it stains a deep red with a 0.01 per cent. aqueous solution of ruthenium red (Brand, 1908, p. 122). Treated with aqueous methylene blue it almost immediately acquires a magenta colour. Chlor-zinc-iodide does not stain the membrane. The pectic layer forms a complete envelope around the contents of each cell, while the surface pellicle extends continuously over the whole plant. The same two layers are distinguishable in the cells of the horizontal system and in the rhizoids. A slight lamellation is sometimes detectable in the pectic layer (Fig. 2 j), especially in the region of the septa. The latter appear plane or concavo-convex in optical section; where cells with plentiful contents adjoin others with a sparser protoplast the convexity of the septum is usually on the side of the latter. The septa always consist of two closely apposed pectic lamellae, although this double nature only becomes apparent under high power examination in most of the vegetative cells. It is then often evident that the pectic layers belonging to adjoining cells are separated by a narrow space so that connexion between such cells is maintained solely or largely by the surface pellicle. This explains the ready fragmentation of the threads at the septa. Remains of the ruptured pellicle are often recognizable at the points of breaking (Figs. 1 A, 1 B, 1 E).

The upper cells of the erect branches, and especially those which become converted into akinetes, undergo an appreciable thickening of the membrane (Figs. 3 C, 4 A, 4 B). The surface pellicle (*c*) becomes more pronounced and the pectic layers (*i*) increase considerably in extent. In such cells the inner pectic part of the wall always shows a very marked lamellation, and thickening is no doubt effected by apposition of successive pectic layers internal to that present in the vegetative cells. All these lamellae stain deeply and in the same way with ruthenium red, and all appear to consist of the same kind of substance.

Special interest attaches to the surface pellicle in these upper cells of the erect branches. Immersion for a period of 60 hours in concentrated sulphuric acid fails to remove it. The pectic layers (including many of the septa) and the cell-contents are dissolved and a similar fate befalls the entire membrane of the rhizoids, horizontal threads, and of the lower parts of the erect threads. The surviving pellicle of the upper parts, after removal of the sulphuric acid, stains quite distinctly, though not very deeply, with Sudan III and Scharlach R. A similar staining is obtained with material subjected to prolonged action of Eau de Javelle, with subsequent washing in 1 per cent. hydrochloric acid. On immersion in 50 per cent. chromic acid the pellicle at once stands out much more clearly and, after several hours, is the only part remaining. After removal of the acid the surviving parts stain deeply with Sudan III and with Scharlach R.

It would thus appear that the outermost layer of the membrane, at least in the upper parts of the erect threads, partakes to a considerable extent of the

nature of a true cuticle, and its special properties indicate a very interesting degree of adaptation to terrestrial conditions. So far as I am aware this is the first time a cuticle has been demonstrated in an alga.<sup>1</sup> When living material of the alga is stained with Sudan III or Scharlach R the surface pellicle becomes more prominent and in the akinetes at least is certainly stained red. It is more difficult to detect a red colour in the thinner pellicle enveloping the ordinary vegetative cells, but it seems not improbable that it may be of the nature of a cuticle over a considerable part of the plant, although sufficiently cuticularized to resist prolonged action of sulphuric or chromic acids only in the upper parts of the erect branches. After prolonged immersion in an aqueous solution of Congo red the surface cuticle of the akinetes is clearly stained, although the inner layers of the membrane do not appear to be affected.

Empty akinetes commonly show a faint longitudinal striation of the membrane (Fig. 4 G), appearing as a considerable number of delicate longitudinal lines which may occasionally anastomose. A similar striation is sometimes indicated in the membrane of vegetative cells which have undergone slight contraction during fixation, although it is not detectable in the living alga.

#### REPRODUCTION

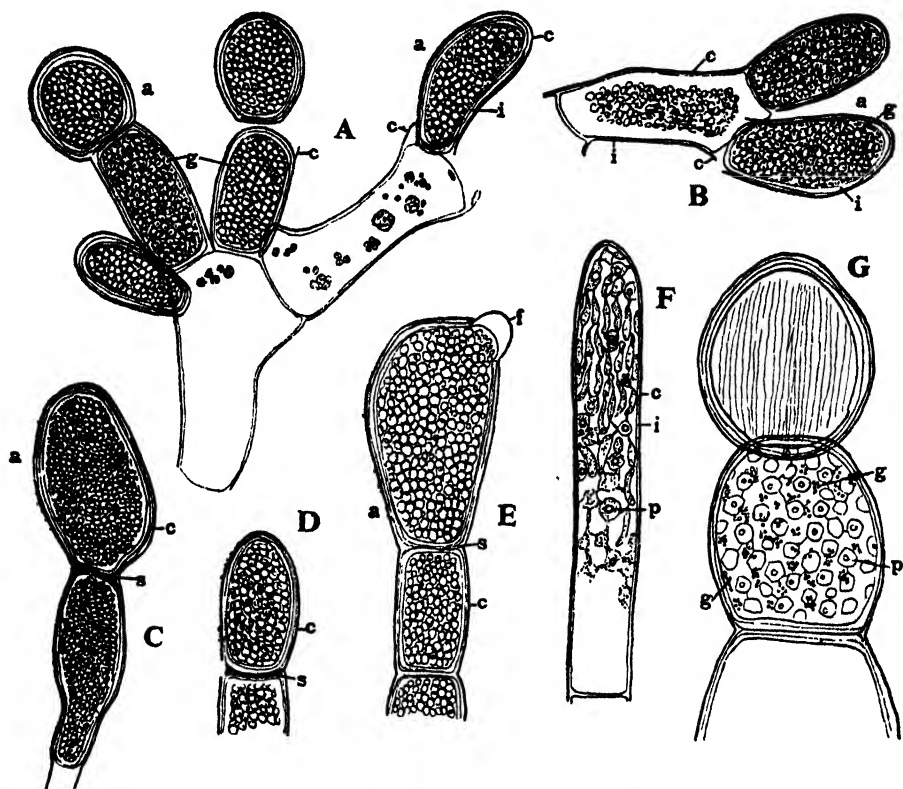
Apart from the vegetative multiplication *in situ* to which reference has been made above, the only other method of reproduction so far observed is by means of akinetes. Although an extensive range of material has been studied and plants have been observed for some time in water or culture solution, no evidence whatsoever of swarmer-formation has been forthcoming. No empty cells with apertures in the wall, such as are typical of swarmer-production in other Cladophoraceae, have ever been encountered.

The bulk of the akinetes are produced from the end-cells of the erect threads and of their branches (Figs. 1 A, 1 B, 1 E-G, a). When these threads are short, the akinetes are usually formed singly or sometimes in pairs from the terminal and subterminal cells. On the other hand, longer threads may form rows of 4 or 5 akinetes (cf. Fig. 4 E). These structures are sometimes produced from cells of the horizontal branches (Figs. 1 D, 1 G), although this is rather exceptional. Intercalary akinetes, formed from an occasional cell within a thread and bounded on either side by vegetative cells, are distinctly rare. Although the akinetes may exhibit the same somewhat elongate cylindrical shape as the vegetative cells (Figs. 1 A, 1 F), they are usually shorter and considerably wider than the latter (Figs. 1 B, 1 E, 1 G); the terminal ones in particular may be almost spherical (Fig. 4 A). Most of the akinetes, however, are oblong in general outline, often with a plane base or one protruded slightly into the underlying cell. They are usually separated by a marked constriction from the neighbouring cells, a feature which may be very marked

<sup>1</sup> According to Blackburn (1936, p. 848) the thimbles in which the cells of *Botryococcus Braunii* are lodged consist of a substance closely related to the cuticular layers of higher plants.

when the akinetes are produced in short rows (Figs. 4 A, 4 C-E, 4 G). The oblong akinetes are mostly  $51-60\ \mu$  wide and  $1\frac{1}{2}-2$  times as long, the spherical ones  $68-82\ \mu$  wide.

The degree of thickening of the membrane varies. Some akinetes have



FIGS. 4 A-4 G. *Cladophorella calcicola* n. gen. et sp. A, B, tips of erect threads with akinetes, in part detached but still *in situ*; two of the terminal akinetes in A are of the spherical type. C, two terminal akinetes separated by a marked split (shown black) in the intervening septum. D, the same, but with the cuticle ruptured on one side. E, part of a chain of akinetes, the top one putting forth a germinal tube (*f*). F, end-cell of a growing erect thread. G, part of a chain of akinetes, the top one empty; in the second the cell-contents are shown in surface-view. *a*, akinetes; *c*, cuticle; *g*, globules (cf. p. 167); *i*, inner (pectic) layers of membrane; *s*, split in septum; *p*, pyrenoids. (A-D  $\times 200$ ; E, G  $\times 300$ ; F  $\times 320$ .)

very thick membranes,  $10-12\ \mu$  in width, although the average thickness is only  $3.5-5\ \mu$ . In the thick-walled akinetes a number of lamellae are recognizable in the wall, sometimes locally separated by narrow interspaces, and each lamella under high power shows an individual stratification.

It seems probable that the akinetes may either become detached as single structures or as short rows which may even be branched. Both conditions are met with abundantly in material that has been subjected to drought and is then mounted in water. Investigation of such material shows that the

septum intervening between two akinetes (Figs. 4 C, 4 D) or between an akinete and an underlying vegetative cell often exhibits an evident split (*s*), and it is clear that when this is so the akinete is attached to the neighbouring cell only by means of the cuticle. Many instances were observed in which the cuticle was ruptured on one (Fig. 4 D) or on all sides (Figs. 4 A, 4 B), although the akinete was still *in situ*. Although such ruptures were probably produced artificially, it can scarcely be doubted that they can also arise in nature and that the freed akinetes can be dispersed by wind-currents. Since, as mentioned above, the vegetative cells underlying akinetes usually exhibit disorganization of the contents (Figs. 4 A, 4 B), it is possible that there may be marked pressure-differences between the two during the maturation of the akinete, resulting in strains that may lead to a weakening and ultimate rupture of the cuticle.

There is no contraction of the contents of the akinetes when material is immersed in 15 per cent. Tidman's sea salt, although healthy vegetative cells in the same material are markedly plasmolysed and their protoplast indeed already shows some contraction in a 5 per cent. solution. The lack of contraction of the contents of the akinetes when immersed in strong hypertonic solutions does not necessarily imply a high osmotic pressure of the contents, since it may be due to impenetrability of the cuticle or to an increased viscosity of the protoplast (Fritsch and Haines, 1923, p. 719). Material with akinetes was allowed to dry on a slide and left exposed to the air of the laboratory for several days. At the end of this period the akinetes appeared quite unaltered and showed no manifest contraction, although the vegetative cells had undergone marked shrivelling. It would thus appear that the akinetes are highly adapted to survive prolonged periods of drought, and the cuticle that envelops them is no doubt of prime importance in this respect.

The cell-contents of the akinetes have already been described above. Iodine solution, which seems to penetrate fairly easily, discloses the presence of large quantities of starch, but apart from this there are commonly present in the peripheral cytoplasm more or less numerous, rather highly refractive glistening globules. Although themselves unstained, they show up very conspicuously against the magenta-coloured inner layers of the membrane when stained with aqueous methylene blue. The globules may be of diverse sizes and rather loosely aggregated, but more usually they are small and of fairly even dimensions. Such small globules may occur, like the larger ones, in loosely disposed aggregates (Fig. 4 G, *g*), but in certain of the mature akinetes they form a semi-continuous peripheral stratum (Figs. 4 A, 4 B, *g*), recalling the dense layer of peripheral globules typical of the akinetes of *Zygogonium ericetorum* (Fritsch, 1916, p. 143). Although the exact nature and function of these structures are still unknown, the occurrence of such peripheral globules in the akinetes of the alga under discussion is of interest. They are, however, by no means always present in considerable quantity, and I am unable to say exactly what determines their occurrence. There is some evidence, however, that they are found particularly in drought-material.

When an akinete becomes detached from the neighbouring cells by



splitting of the septum and rupture of the cuticle, the latter probably develops also over the exposed surface, although I am not able to say how this takes place in detail. When sulphuric acid is drawn in to material with abundant detached akinetes mounted in water under a cover slip, penetration of the acid is marked by the cell-contents assuming a light-green colour; very soon after that the pectic layers swell and these, together with the contents, flow out through a break in the cuticular envelope, often leaving it in great part empty. Such empty cuticles display irregular cracks, exposing a wide rupture through which the swelling contents have pushed their way. On the other hand, the cuticle of occasional akinetes retains the original form; in such instances there is a more or less wide circular aperture at one end through which the contents have escaped, and this no doubt marks the position of a septum which had not developed a cuticle.

There can be no doubt that, when several contiguous cells become transformed into akinetes, they generally remain as a coherent entity, although the pectic layers of the membrane are double at the septa (Figs. 4 A, 4 E) and sometimes show indications of a split (s). Such rows of cohering akinetes, in which all or some were in process of germination, were frequently encountered. The factors causing detachment of individual akinetes require further investigation.

Germinating akinetes were met with plentifully amid the dense growths of the alga, although I have been unsuccessful in bringing about such germination in cultures. The process commences with protrusion of the contents surrounded by an inner pectin lamella through the ruptured cuticle (Fig. 1 H, f). Such outgrowth can apparently ensue at any point on the surface of the akinete (cf. Figs. 2 E, 2 F, 4 E, f), although the germinal tube commonly arises at one end (Figs. 2 C, 2 D). The whole of the contents of the akinete may pass into the outgrowing filament leaving it empty (Figs. 2 C, 2 D, 2 F, 2 G) or occasionally a part remains behind and becomes separated by a septum from the rest of the germinal tube (Fig. 2 E). The latter may attain an appreciable length before septation occurs (Figs. 2 E, 2 F); in such instances the greater part of the germinal tube is generally empty, with the contents massed towards the distal end and especially densely aggregated beneath the growing tip. In other instances (Fig. 2 G) septa are formed at an early stage. Branching may occur before the young filament has reached any considerable length (Fig. 2 D), although usually deferred to a rather later stage (Fig. 2 c).

The germination stages just described were found among the tufts of the mature alga, and I suspect that the marked elongation of the germinal tube is in response to the necessity of bringing the germling to the light. Germination of akinetes under conditions of lesser competition may well take place somewhat differently, with an earlier appearance of septation and earlier branching. I am at present unable to say exactly how the horizontal system originates, although occasional germlings (Fig. 2 H) displayed in their upper parts an incipient production of short horizontal outgrowths which may well be the starting-points of the creeping threads.

# AFFINITIES

The cell-structure (lamellate membrane, reticulate chloroplast with many pyrenoids, several nuclei per cell) shows that the alga above described is a member of the Cladophorales. The erect threads resemble in many respects those of a small Cladophora, although differing from those of the majority of its species in the frequent origin of the branches at some little distance beneath the upper septum of the parent cell. This is a feature met with in the species placed in the subgenus *Aegagropila*, as well as in some species of *Pithophora*. The frequent formation of the first septum in the branch at some distance above its base, although slightly indicated in some Cladophoraceae, is never as marked as in *Cladophorella*. In its apical growth and calcicole habit the latter agrees with several members of the order.

The general habit contrasts markedly with that of other known Cladophorales. The erect branches arise from a well-marked horizontal system of large cells, some of which are produced into elongate attaching rhizoids. It is probable that the horizontal system is not primary, but that it arises secondarily as a branch of the primary filament produced from an akinete (cf. above). It is thus not a true heterotrichous system, such as appears to occur in *Arnoldiella* (Miller, 1928; Fritsch, 1935, p. 245) which shows some points of contact with *Cladophorella*. In *Arnoldiella* there is a first-formed filamentous basal stratum directly attached to the substratum. A somewhat similar stratum occurs in Skuja's *Cladostroma* (1937, p. 81), where, however, the growth seems to be almost entirely prostrate, with only the tips of the branches bending up. *Arnoldiella* and *Cladostroma* stand apart from the main series of the Cladophoraceae and their exact affinities are at present dubious.

The distinctive habit, as well as the terrestrial mode of life, warrant the establishment of a distinct genus for the alga described in the preceding pages. The only other terrestrial member of the order is *Wittrockiella* (Wille, 1909), which grows on soil and differs in many important respects. Its relation to other Cladophorales is very obscure and both for it, as well as for the two genera mentioned in the preceding paragraph, the cell-structure may not give a true index of the actual affinities. On the other hand, it is not too difficult to interpret *Cladophorella* as a *Cladophora* or *Pithophora* which has become modified in relation to a terrestrial habitat. The minute size of the plant is paralleled in certain aquatic Cladophoras (e.g. *C. inconspicua*, West, 1907, p. 103), where, however, the short dense tufts are entirely erect. Species of *Cladophora* are known to be capable of surviving under subaerial conditions (Heering, 1921, p. 14). The faculty of producing elongate attaching rhizoids from the cells of the main axes is widespread among the species of *Cladophora* where, however, these axes grow vertically and not horizontally. The formation of horizontal creeping branches is probably a direct adaptation to growth on the rock-surface. The frequent irregular shape of the branch-producing cells of the horizontal system is met with also in some of the *Aegagropilas* (Heering, 1921, fig. 47).

The features associated with the production of akinetes relate the alga both to *Cladophora* and *Pithophora*. The frequent practical emptiness of the cells underlying mature akinetes, which is almost certainly a result of the passage of the greater part of the cell-contents into the latter before they are cut off, recalls the mode of akinete-formation characteristic of the latter genus. On the other hand the formation of akinetes in chains, in which the individual structures tend to remain coherent, is comparable to the condition in which many *Cladophoras* pass through the dormant season. If, however, the new alga is rightly interpreted as a tropical form, a nearer relation to *Pithophora*, which is widely represented in tropical freshwaters, is perhaps to be suspected. The relatively thin walls of the vegetative cells and the mode of branch-production speak for such an affinity, as also does the apparent absence of methods of reproduction other than by fragmentation and akinete-formation.

#### SUMMARY

*Cladophorella calcicola* nov. gen. et sp. is a terrestrial member of Cladophoraceae inhabiting calcareous (oolitic) rock-fragments in the damper hot-houses of the Cambridge Botanical Gardens, though lacking on similar substrata outside. The plants consist of a complex system of large-celled horizontal threads, readily becoming detached from one another at the septa, and attached to the substratum by elongate rhizoids which are usually unbranched and devoid of septa. The usually narrower erect threads arising from the horizontal ones form dense tufts reaching a height of 2-5 mm. The often numerous branches arise laterally, commonly some little way beneath the upper septum of the parent cell, and the first transverse wall in the branch is frequently situated some distance above its base. The vegetative cells generally have a relatively thin membrane, contain a reticulate chloroplast with numerous pyrenoids, and internal to it a limited number of nuclei. The membrane consists of a pectic layer bounded by a surface cuticle.

Multiplication is effected by fragmentation *in situ* and by akinetes with dense contents harbouring numerous large pyrenoids. For the most part these are produced terminally on the erect threads, either singly or in series. The akinetes have a thick lamellate membrane, the surface layer of which shows many of the characteristics of a true cuticle; for instance it is unaffected by 60 hours' immersion in concentrated sulphuric acid. It is not improbable that the surface layer of the wall is cuticularized over most of the erect branches, though less markedly so in the lower parts.

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# Experimental and Analytical Studies of Pteridophytes

## III. Stelar Morphology: The Initial Differentiation of Vascular Tissue

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With Plate III and nine Figures in the Text

### I. INTRODUCTION

IN these studies an attempt will be made to examine critically the present state of knowledge of the vascular systems of pteridophytes and the operation of some of the factors which appear to be causally related to their inception and development. A particular interest should attach to a comparison of such conclusions as may be reached with those which emerged and were considered valid during the period when phyletic studies were in the ascendant and when morphology and physiology were pursued almost as separate branches of botanical science.

The observations now presented refer to the initial differentiation of vascular tissue.

### II. BRIEF REVIEW OF STELAR INVESTIGATIONS

In opposition to the views of earlier workers who regarded the *individual strand* as the unit of vascular construction, van Tieghem (1886) recognized the *vascular system*, whether compact and simple or disintegrated and complex, as the unit. This he called the stele. It was a conception which, like one that had already been formulated by Sachs, tended towards a recognition of the essential unity of the shoot. Certain details of van Tieghem's theory proved untenable in the light of further investigation, but the service which he rendered is unmistakable. The steps in the increasing complexity of stelar structure, as outlined by Gwynne Vaughan and others, included a recognition of the protostele as a primitive type to be encountered in the adult shoots of certain ferns regarded as ancient and primitive, and at the base of the young plant in types which attain to greater stelar elaboration in the adult state and which, from a consensus of evidence, are regarded as recent and derivative. In the course of the individual development the simple protostele may become medullated, a condition which leads to solenostely, and this in turn, in shoots where the foliar-gaps overlap, to dictyostely. In the latter the stele typically consists of a network of conducting tissues and in transverse section is seen as a ring of separate meristeles. To these developments may be added the instances of 'perforation' where the vascular mesh-

work is further elaborated but not necessarily in relation to foliar-gaps, and of polycycly, where two or more concentric rings of vascular tissue are present. The relation of the vascular strands of the leaves, i.e. the leaf-traces, to the shoot stele has also been extensively explored. Full accounts of these several developments with citations of the relevant literature are given by Bower (1923) and Ogura (1938).

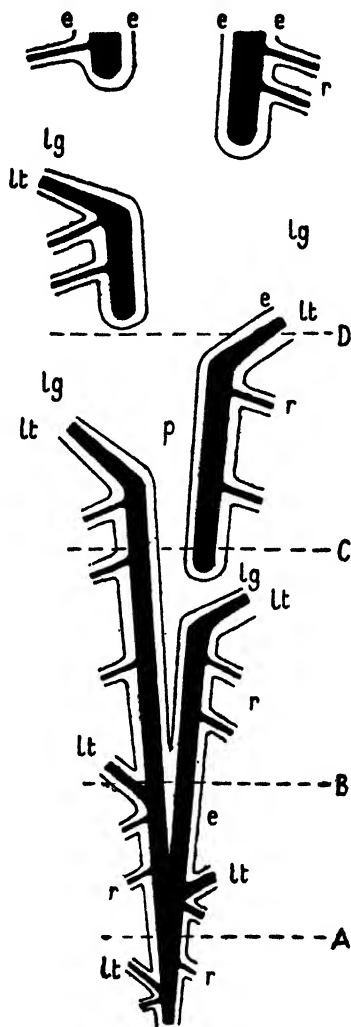
These several investigations were almost entirely morphological in their inception and outlook; physiological aspects were left practically untouched and in fact remain an unworked field. So, too, records of experimental investigations are of very occasional occurrence and it is only during recent years that tentative causal views regarding the observed diversity of stelar structure have been propounded. Considerations of space do not permit of a critical discussion of the various theories and speculations which, labouring under the handicaps indicated above, have at one time or another been advanced to account for the observed facts of stelar morphology, but some of these, to which further reference will be made in this series of papers, are: (i) that the several vascular strands which may be present in a shoot constitute a single unit of construction, the stele; (ii) that, in different instances, leaves and shoot have proved of very different relative importance in contributing towards the development of the vascular system; (iii) that the pith represents an intrusion of the cortex into the stele; (iv) that the vascular system affords a useful criterion of comparison in phyletic studies; (v) that, in its development, the vascular system shows changes which may be interpreted as being adaptive and proportionate to functional requirements; (vi) that during development a 'change of destination' may in some instances be observed in the differentiation of the tissues of the procambial strand; (vii) that the vascular system lends itself to interpretation in terms of causal factors, the size-factor being of particular importance.

### III. A NOTE ON METHOD

In the elaboration of the views indicated above, the method of investigation consisted for the most part in the examination of transverse sections of the shoot from the base upwards, or more locally through one or two nodes only. The inadequacies of the interpretations undoubtedly issue from the limitations of the method used. Reference to the extensive literature of pteridophyte stelar morphology brings out the fact that by far the greater number of memoirs—including those which may now be considered classics—are devoted almost entirely to anatomical considerations of fully differentiated tissue systems usually in adult regions of the plant (cf. Ogura, 1938). The limitations of such an approach now seem evident. It was realized, of course, that in the obconical development from the young sporeling stage to the fully expanded adult shoot, all the stages in the ontogeny of the individual vascular system could readily be traced; such studies, in fact, provided a more or less complete picture of the progressive increase in complexity

during development. Thus, by way of illustrating a characteristic morphological argument, the protostele at level *A* in Text-fig. 1 is the condition to be encountered in the young sporophyte; the condition at level *B* has been 'derived' from that at level *A* by medullation of the stele; the condition at level *C* from that at level *B* by the development of considerable leaf-gaps, &c., and the condition at level *D* from that at level *C* by the overlapping of leaf-gaps. Now there is a sense in which this kind of description is both acceptable and useful: it indicates a continuous system of relations during development. But in point of fact, level *B* is not directly developed or derived from level *A*, nor level *C* from level *B*, though in each case the antecedent phase may be considered to influence that which follows it. The condition of the fully differentiated tissues at level *A*, to be precise, is primarily referable to the size, activity, and nutritional status of the apex at the time of inception of that stage; and similarly for level *B*, and so on. It would appear that investigations along these lines have not hitherto been pressed, and hence information essential to a critical interpretation of stelar structure and making for further fruitful investigation has been lacking. The leaf-trace likewise needs to be studied, not merely in adult regions of the plant, but during the earliest stages of its development. At some later date accounts of the phenomena under consideration may perhaps be formulated in physiological terms; an essential preliminary condition is that the basic morphological facts should be adequately investigated. The common assumption that these have already been ascertained is erroneous.

A further criticism relates to observations and ideas current in morphological literature, in which it has become almost a normal procedure to consider the vascular system apart from the associated cortical tissues, i.e. as if it had an existence of its own—a defect which is traceable to van Tieghem's original



TEXT-FIG. 1. Diagrammatic plan of the stelar structure in a dictyostelic fern from the base into the adult region of the shoot. *r*, root; *lt*, leaf-trace; *lg*, leaf-gap; *p*, pith; *e*, endodermis. Xylem is shown black, phloem and stelar parenchyma lie in the unshaded region between endodermis and xylem. *A*, *B*, *C*, *D*, levels of development discussed in the text.



stelar theory. Many of the published reconstructions of steles (like Text-fig. 1, for example) afford no indication of the relation between the vascular system and the outline of the shoot as a whole. By thus abstracting the stele from its enveloping tissue, it is inevitable that important aspects of the development and organization of the shoot system have been neglected.

Thus while a study of the vascular system from the base of the plant upwards into the adult region provides valuable information on the final condition, the factors operative during development can only be assessed if information relating to the differentiation and development behind the apex, not only of the stele but of all tissues, is obtained for all stages during growth to the adult, i.e. *the development of the shoot as a whole should be investigated from the apex backwards at successive stages during the ontogeny.*

#### IV. HYPOTHESIS REGARDING THE INITIAL DIFFERENTIATION OF VASCULAR TISSUE

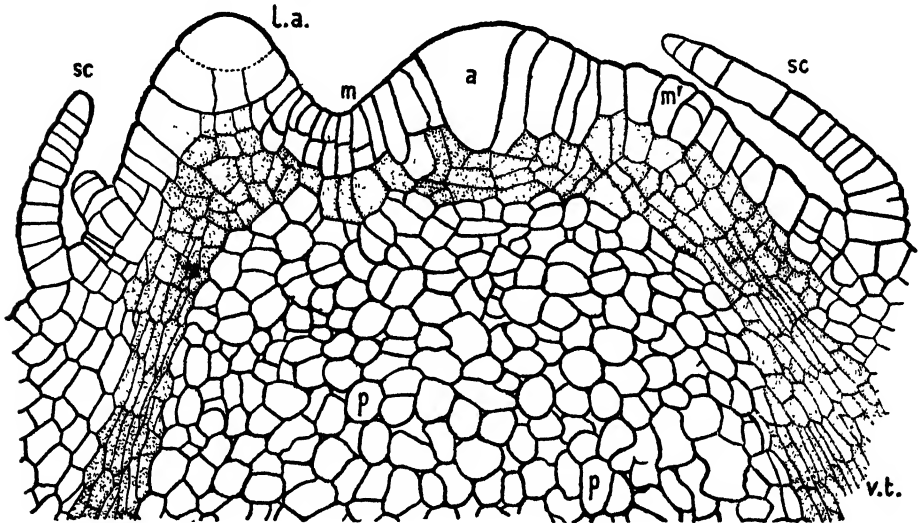
*Need for a working hypothesis.* In order to investigate dynamic aspects of the development and elaboration of the stelar system it is essential, as a first step, that there should be a clear understanding of the factors responsible for, or causally related to, the *initial differentiation* of vascular tissue at the shoot apex. In fact, singularly little is known of this either from direct experimental observation or inferentially from other data. There can be little doubt that the absence of such knowledge has been a serious obstacle to progress in the investigation of stelar problems. An immediate requirement is to consider the possibility of formulating a working hypothesis regarding the initial differentiation of vascular tissue which will account for all the facts so far known from direct observation. Such an hypothesis should prepare the way for experimental investigations.

In 1915 Lang pointed out that a deeper insight into the nature of the stele may be obtained by regarding it as 'the resultant of a number of factors, as part of the manifestation of the system of relations in development'. He further remarked: 'possible influences that have at various times been suggested are functional stimuli, the inductive influence of the older pre-formed parts on the developing region, and formative stimuli of unknown nature proceeding from the developing region.' Of these possibilities the third commends itself as deserving special attention. It might have been anticipated that this stimulating lead would have made for progress in the investigation of the apical region and have been productive of new concepts. As far as the pteridophytes are concerned this does not appear to have been the case.

In the development of vascular tissue two main phases may usefully be distinguished: (i) the *initial differentiation* whereby vascular tissue can be distinguished from cortical tissue, and (ii) the *subsequent differentiation* characterized by the specialized development of the constituent vascular

elements. It is in the relatively brief region situated *immediately below* the apical meristem that the *initial differentiation* takes place. It is cogent to inquire therefore what relationship, if any, may be considered to exist between the activity of the apical meristem and the initial differentiation of vascular tissue.

*Metabolism at the apical meristem.* Formative processes at the apical



TEXT-FIG. 2. *Dryopteris aristata*. Longitudinal section through the terminal apex of a small shoot, showing the apical cell *a*, the extent of the apical meristem *m-m'*, and the vascular tissue *vt* in the initial state of differentiation lying immediately below the apical meristem. *l.a.*, leaf apex; *sc*, scale; *p*, pith. ( $\times 158$ .)

meristem involve as a first consideration an increase in the amount of protoplasm, i.e. the meristem is a region of active protein synthesis. From the complex of biochemical processes involved, enzymes and other substances, e.g. auxins, possessing important physiological properties are known to result. These will tend to diffuse or otherwise move from the region of highest concentration—the meristematic cells in which they are produced—into the adjacent cells, and these, as also those at a distance, are therefore liable to undergo changes in their physiological and structural properties. Now, in leptosporangiate ferns the first observable differentiation of vascular tissue takes place *immediately* below the apical meristem. The latter consists of a single distinctive layer of densely protoplasmic cells: these divide by periclinal walls and give rise to vascular tissue in its initial state, as in Text-fig. 2. (Text-figs. 2–6 were obtained by tracing over photographic prints.)

In shoot apices fixed whilst in an *actively growing condition* this *initial differentiation* is readily apparent in suitably stained sections (safranin and Delafield's haematoxylin). It is, therefore, tentatively suggested that this *initial differentiation* is attributable to the diffusion of a substance (or sub-

stances) from the meristematic cells. Such a view gives greater definition to the conception of 'formative stimuli of unknown nature proceeding from the developing region'.<sup>1</sup> The relationship under consideration is illustrated for various pteridophytes in Pl. III. The disposition of the cells of the terminal meristem is such as to make for the diffusion of substances in a centripetal and downward direction. Since there is also a contemporaneous movement of nutrients of various kinds *towards* the apex from below, the region of interaction extending downwards from the region of initial differentiation is characterized by further notable histological changes, i.e. those constituting the phase of *subsequent differentiation*. A further source of interaction in the developing region, which is known to be characterized by a high rate of respiration, may relate to the gradients of moisture, oxygen, and carbon dioxide concentrations between the centre of the shoot and the external atmosphere.

*Statement of working hypothesis.* A working hypothesis regarding the initial differentiation of the vascular tissue in pteridophytes may now be stated as follows. *Wherever the apical meristem of a shoot, bud, leaf, or root is in a state of active growth of such a nature that the distinctive character of the meristematic cells is maintained, the initial differentiation of vascular tissue will be observable immediately below the apex and in the path of substances diffusing from it, one or more of these substances being causally involved in that process.* What the diffusing substance (or substances) may be and how it works is not known, but a clear statement of the problem should prepare the way for experimental investigations. In the meantime it is cogent to inquire to what extent the hypothesis is supported by the available data (Section V).

The hypothesis indicates that the maintenance of the distinctive character of the meristematic cells is important. Here the writer has in mind instances where the apex may still be in a state of growth for some time but where the meristem gradually disappears by being transformed into a region of distended parenchymatous tissue. In these instances the active formation of protoplasm is evidently at an end; an attendant condition is that vascular tissue ceases to be differentiated (Section V). The general problem under consideration therefore also requires the elucidation of another important physiological

<sup>1</sup> The fact that new protoplasm is being formed at the apex does not show conclusively that protein synthesis occurs in the terminal meristem or in the terminal meristem only. The inference is probably justified, but little if anything is known of the seat of protein synthesis, and although it is not improbable that amino-acids are supplied to the apical meristem, it is just possible that more complex substances such as metaproteins, peptones, and proteoses can diffuse across living tissues and may therefore form the basis for protein metabolism in the meristem.

Again, the statement that auxin arises in meristematic cells might be questioned. It is known that it is formed in embryonic tissues like buds, but whether it is formed in the apical meristem is another matter.

While the evidence appears to demonstrate the dominating influence of the apical meristem on initial differentiation, this influence may also be due not to the diffusion backwards of particular substances but to other mechanisms. For instance, it is possible that activity in the meristem involves the withdrawal of substances from the tissues below it, and it may be the removal of materials instead of their application that conditions initial differentiation.

process, that by which active distal meristems are supplied with nitrogenous metabolites, some of which may be of considerable molecular complexity, by way of undifferentiated vascular tissue.

*Initial histological condition during ontogeny.* In young sporophytes and in small buds the apical meristem is relatively inextensive and the diameter of the differentiating vascular tissue is small. In adult plants the apical meristem is more extensive and the associated vascular tissue is of correspondingly large amount. Both in the young sporophyte and in the adult plant the stele, in the initial phase of differentiation as defined here, consists of a coherent, uninterrupted, homogeneous tissue. Even though this phase may be of very brief spatial extent, as in the shoot apices of adult dictyostelic ferns, it has, nevertheless, a real and demonstrable existence. Thus the initial phase of stelar differentiation is one which remains essentially unchanged throughout development, the observable changes being quantitative, i.e. an increase in the diameter of the stele. On the other hand, where the apical meristem becomes smaller, as a result of 'starvation' or other experimental treatment, there is a corresponding reduction in the diameter of the stele during the initial phase of differentiation and subsequently in its size and configuration when fully differentiated.

In the *subsequent differentiation* of the stele many factors are operative. Collectively they determine the diversity in anatomical and structural development to be observed in a single individual on proceeding from base to apex. The nutritional status of the organism as determining the supply of nutrients to formative regions is of primary importance.

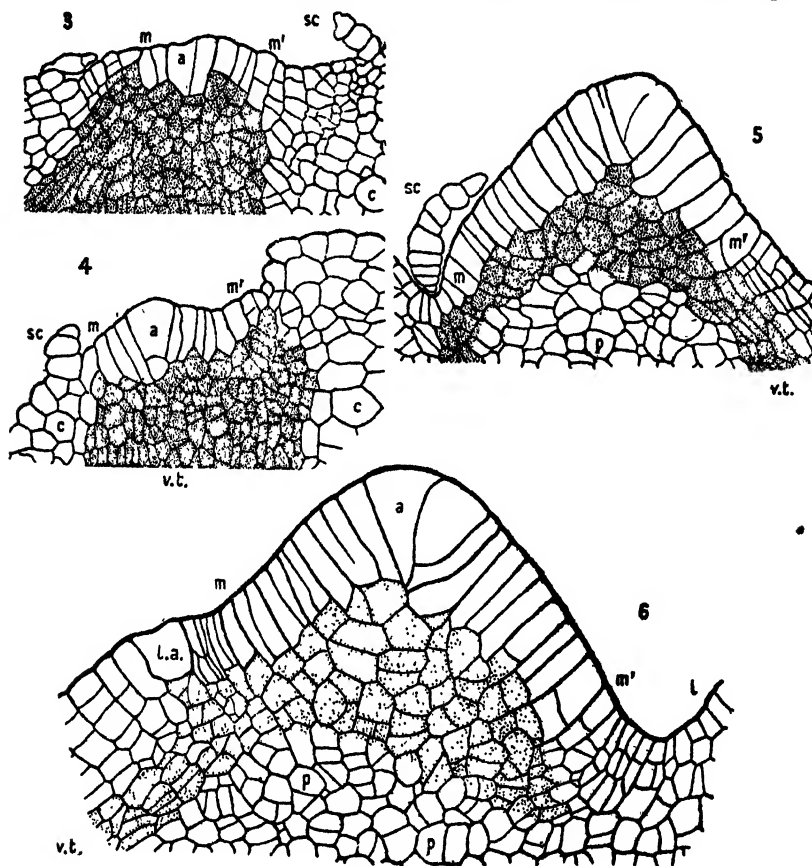
*Vascular systems of leaf and root.* The relationship under consideration should also hold good for leaves and roots. As the initial differentiation of the vascular tissue of a leaf primordium is approximately contemporaneous with the initial differentiation of the adjacent shoot stele, a coherent and unified vascular system results. The leaf-trace in fact, 'passes' backwards *into the shoot*, not *out from the shoot* as is sometimes described in anatomical investigations based on adult regions. This is a point to which Lang (1915) has called attention.

## V. SUPPORTING EVIDENCE

Tests of the sufficiency of the hypothesis should include: (i) demonstration of a close and invariable relationship between the presence of an active apical meristem and the initial differentiation of vascular tissue in all classes of pteridophytes, and (ii) evidence of the presence or absence of such differentiation in relation to the presence or absence (or disappearance) respectively of an apical meristem. Final verification will require the experimental induction of the initial phase of differentiation in non-vascularized tissue by the application of substances derived from active meristems. So far as the writer is aware no such work has yet been attempted.

*General evidence.* In pteridophytes generally, it is a fact of observation that wherever an actively growing apex is present in shoot, leaf, or root, vascular

tissue may be observed in the process of differentiation immediately behind it. Moreover, the transverse dimensions of the newly differentiated tissue are closely related to the extent of the apical meristem; Text-figs. 3-6



TEXT-FIGS. 3-6. *Matteuccia struthiopteris*. Longitudinal sections through small and large shoot apices, showing the relation of the vascular tissue, in its initial phase of differentiation, to the apical meristem. Fig. 3, very young rhizome emerging from erect shoot. Fig. 4, apex of young plantling; Fig. 5, apex of young erect shoot, one year old. Fig. 6, apex of older erect shoot. In Figs. 3, 4, 5, the dense stippling indicates the easily observable vascular tissue below the apical meristem; in Fig. 6, the lighter stippling indicates, tentatively, the position of vascular tissue; this specimen was collected after cessation of growth in late autumn and did not respond to the differential staining as did the materials of Figs. 3-5. a, apical cell; m-m', apical meristem; v.t., vascular tissue; p, pith; sc, scale; l.a., leaf apex; l, leaf. ( $\times 135$ .)

illustrate this fact for the ostrich fern (*Matteuccia struthiopteris*). (See also Pl. III, Figs. 1, 2, 3, 5, 7.)

*Inactive and active meristems.* From an examination of fern shoot apices collected throughout the year, e.g. those of *Matteuccia struthiopteris*, it has been ascertained that whereas during the season of active growth the initial differentiation of the vascular tissue immediately below the apices of shoot

and leaf primordia is readily apparent in double-stained preparations, such differentiation cannot be observed during the season of quiescence (Text-fig. 6). Valuable data should accrue from further detailed studies of fern apices throughout the year.

In the rhizomatous shoots of *M. struthiopteris* and *Onoclea sensibilis* groups of meristematic cells become separated from the apical meristem and occur in specific positions along the rhizome as *inactive detached meristems* (Wardlaw, 1943, *a*). In the normal course of development the only contribution to the enlarging shoot made by these meristematic cells consists in the formation of a few layers of parenchyma on their inner side. But immediately such detached meristems are induced to begin development into lateral shoots by the removal of the terminal apex, the differentiation of vascular tissue also begins. This may or may not become conjoined with the stele of the parental shoot, depending on the distance of the bud from the shoot apex; Pl. III, Figs. 4, 6. Data relating to the development of bud primordia in other ferns are in close agreement. In species of *Dryopteris*, experimentally induced lateral buds have been observed (i) in complete vascular continuity with the shoot stele when development took place close to the terminal meristem, (ii) in incomplete continuity when further removed from the terminal meristem, and (iii) not in vascular connexion with the shoot stele, Pl. III, Figs. 8, 9 (Wardlaw, 1943 *a*). Buds of the third category have an important bearing on the hypothesis under consideration, since in these instances the direct influence of the vascular system of the parent shoot on the initial differentiation of the bud stele can be ruled out. In such buds the initial differentiation can be observed immediately below the actively developing meristematic cells of the newly constituted apex. The cortical parenchyma of the parent shoot in proximity and even some distance away may also be affected, the cells showing a succession of divisions into the small-celled tissue characteristic of the stele in its initial phase of differentiation. Finally the bud stele tapers out and ends blindly in the cortex of the parental shoot. That the observed histological changes are related to the diffusion of a substance or substances from the active bud meristem seems highly probable, though other factors are also involved.

The young sporophytes of certain species of *Lycopodium* are characterized by the development of a distended, starch-storing organ, sometimes known as the protocorm, which organographically occupies the position of the shoot and bears small leaves. Each leaf, as a rule, has a vascular strand which ends blindly in the protocorm. Up to a certain stage the protocorm has no perceptible or distinctive formative apex and no vascular tissue is differentiated. But later, an actively growing apex can be observed, and behind it there develops a vascular strand. It is proposed to consider these facts in greater detail at a later time. Other instances of a similar kind are known for other pteridophytes.

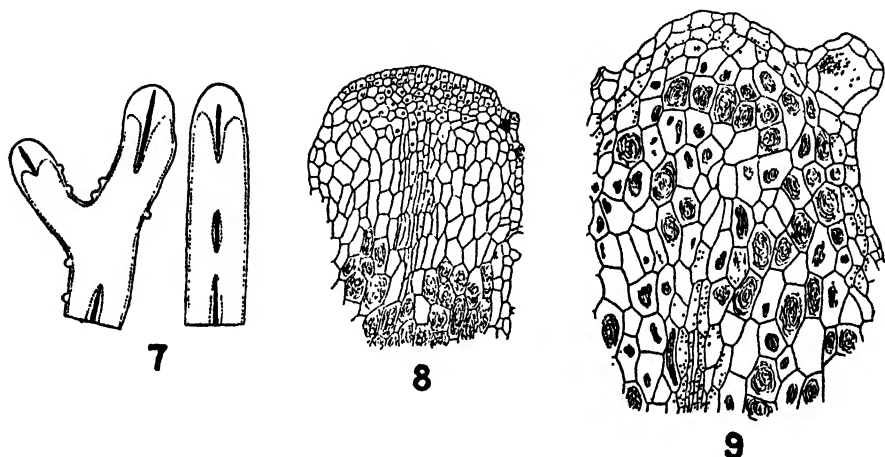
*Discontinuous apical activity.* Holloway (1939) has described large prothalli of *Psilotum triquetrum* in which a vascular strand may be present. The point

of special interest for the present purpose lies in the fact that this vascular strand may be discontinuous, i.e., it appears and disappears at intervals along the length of the approximately cylindrical prothallus. The prothallus is entirely saprophytic and subterranean; mycorrhiza are abundantly present and starch grains occur as a normal storage deposit. Small prothalli, those found growing in flaky rock, do not develop a vascular strand; the larger prothalli are typically obtained from soil. When a vascular strand is present it originates immediately behind the growing apex, this being a region of small, dividing cells with dense protoplasmic contents. Near such an apex, as also in peripheral regions and in the vascular strand, starch grains and mycorrhizic filaments, though present in adjacent parenchymatous tissue, are not in evidence. Periodically, in relation to seasonal or other conditions not precisely indicated, formative activity at the apex ceases; a majority of the cells constituting the meristem become enlarged into typical parenchyma and starch is deposited in them and they become invaded by mycorrhizal filaments; moreover, the differentiation of vascular tissue ceases. In other words, a carbohydrate and mycorrhizic phase appears to supervene upon the previous meristematic phase. A reasonable inference is that the substance (or substances) diffusing from the apical meristem in its active condition and relating to (i) the differentiation of vascular tissue, and (ii) the absence of mycorrhiza in cells, is no longer being produced in critical concentration. After some time, again in relation to conditions not specified, there is a renewal of meristematic activity in the most distal, densely protoplasmic, and least enlarged cells, and again a vascular cylinder becomes differentiated, adjacent regions showing the characteristic absence of mycorrhiza. Here then is evidence of a direct relationship between meristematic activity and the differentiation of vascular tissue (Text-figs. 7, 8, 9).

Somewhat similar evidence has been obtained in leptosporangiate ferns when the apical cells of the shoot or of leaf primordia have been damaged. The subsequent development has consisted in the formation of a uniform region of parenchyma, and the differentiation of vascular tissue has ceased.

*Other instances.* In his study of regenerative growth in *Lycopodium selago*, Williams (1933) has described a number of structures to which reference may be made. These include (a) organs described as 'protophylls', (b) coralloid growths, and (c) abnormal apices. 'Protophylls' are awl-like regenerative outgrowths of epidermal origin; they possess numerous stomata and consist internally of a spongy mesophyll, but evidence of a formative apical region or of vascular tissue is entirely lacking. Only in the swollen basal region is there evidence of some tracheidal development. Coralloid growths are obtained when proliferating (regenerating) epidermal and subepidermal regions fail to develop stem apices. The development of proliferating cell masses is very slow during the winter period, but thereafter active growth results in the development of coralloid, i.e. lobed, dark green structures. These growths consist of large, vacuolated cells containing abundant starch grains surrounded by a superficial layer of small cells, 'the product of numerous centres of

meristematic activity'. Williams observes that 'apart from a small group of reticulately thickened tracheidal cells in the primary cell mass, the coralloid growths were entirely devoid of vascular tissue'. Here, apparently, a phase characterized by vigorous carbohydrate metabolism has supervened on the 'normal' meristematic activity of the potential shoot, and differentiation of vascular tissue has failed to take place.



TEXT-FIGS. 7-9. *Psilotum triquetrum* (after Holloway). Fig. 7. Diagrams drawn to scale of the forward portions of two large gametophytes in longitudinal view, showing the discontinuous conducting strand and also the distribution of the fungus. ( $\times 10$ .) Fig. 8. Median longitudinal section of a large gametophyte in the active condition, showing the apical origin of the conducting strand. ( $\times 50$ .) Fig. 9. Median longitudinal section of another gametophyte showing the apex in the inactive condition; no vascular strand is being formed. ( $\times 50$ .)

Lastly, Williams has described certain anomalous apices in which the distal region becomes bulbous and fails to form the normal succession of leaf primordia. Such apices show no evidence of the normal small-celled meristem, the arrested leaf rudiments and apical region consisting of large cells; the differentiated axial stele ends at the level of the highest normal leaf; above that level there is an abnormal procambial strand which ends some distance below the tip of the stem.

Collectively, this evidence indicates that in those instances where the apical growth, whether of shoot or leaf, is not of such a nature that the distinctive character of the meristematic cells is maintained, stelar tissue is not differentiated.

## VI. DISCUSSION

Only a few of the issues raised in the present paper can be discussed in detail here. In the preceding section it has been seen that a considerable body of evidence directly relevant to the hypothesis can be adduced; in fact, various consequences of the hypothesis have been observed. The way has



therefore been prepared for the experimental investigation of a major problem—the initial differentiation of vascular tissue. If a direct relationship between the diffusion of a substance (or substances) from active cells of the terminal meristem and the initial differentiation of vascular tissue can be established, a significant advance will have been made in the investigation of formative processes at the apex, and the possibility of a more fundamental interpretation of structural and morphological development greatly enhanced.

In pteridophytes the relative importance of the shoot and of the leaf in contributing to the vascular system has in the past been considered at some length. Some writers have maintained that the shoot stele is essentially of axial origin, others that it is a composite structure built up from the decurrent vascular strands from the leaves. Evidence has been adduced in support of both points of view and there is no doubt that, in different instances, significant differences in the relative developmental activity of shoot and leaf do occur (cf. Bower, 1923, p. 139). The hypothesis now presented, however, is not only equally applicable to both sets of data but serves to unify them, i.e. the two conceptions are seen to refer to variants of the same morphogenic process. Thus, active shoot growth which results in the formation of an elongated rhizome will be productive of an undeniable cauline stele: on the other hand, active leaf development and relative inactivity at the shoot apex may be productive, at least for a time, of a vascular system which will appear to be a composite structure consisting largely of decurrent leaf-traces with little or no cauline component. As the differentiation of vascular tissue behind the shoot apex and behind young leaf primordia takes place contemporaneously and in close proximity, the production of a unified vascular system naturally results.

Some reference must be made to the bearing of the hypothesis on the initiation of primary vascular tissues in seed plants—recently the subject of an extensive review by Esau (1943). This useful survey affords a clear impression of the prevailing confusion of ideas and terminology and of the extent to which the subject has been elaborated in terms of almost purely anatomical concepts. As the writer has already pointed out (1943 *a*), the shoot apex must be studied *as a whole*; any particular developing region, e.g. the terminal meristem, a leaf or bud primordium, should be investigated not as an isolated object but as an inseparable part of an integrated dynamic system. The literature concerning apical meristems and the initial differentiation of vascular tissue in different families of dicotyledons and in gymnosperms indicates considerable histological diversity. The leptosporangiate ferns, on which the writer's investigations are mainly based, appear to afford particularly favourable materials for the investigations under consideration, since the *apical meristem* consists of a precisely definable region composed of a single superficial layer of distinctive and easily recognizable cells. This *apical meristem* would apparently coincide with the 'Urmeristem' ('promeristem') of Nägeli (1858). In actively growing leptosporangiate ferns vascular tissue in the initial phase of differentiation can be distinguished

immediately below the apical meristem, whereas in dicotyledons this phase is more obscure and very different interpretations of the histological details have been put forward. Thus Nägeli recognized a region of partly differentiated meristem ('Folgermeristem') in which the cambial strands are embedded. Other workers have described a 'thickening ring', a 'cambium ring', a 'méristème prévasculaire', a 'primary meristem ring', a 'prodesmogen', a 'Restmeristem' ('residual meristem') situated below the terminal meristem; from this 'ring' the procambial strands apparently become differentiated.<sup>1</sup> Among modern investigators, such as Avery (1933) and Foster (1942), terms such as 'provascular tissue' and 'provascular meristem' have been used. Priestley (1928) has stated that in many dicotyledons the procambial elements arise below the apex as a continuous ring, the subsequent isolation of bundles resulting from a development of parenchymatous tissue from parts of the procambium ring. While Helm (1931, 1937), Louis (1935), and Kaplan (1936, 1937) are in agreement that the vascular system of shoots does not arise directly from the 'promeristem' but in a highly meristematic region situated below it and in relation to the development of leaf primordia, Esau (1943, p. 141) holds the view that 'the value of interpolating a special distinct phase between apical meristem and procambium is yet to be tested by means of detailed histogenetic studies'. The new and additional terminology coined by Helm also seems to be of doubtful value. That differences between the terminal meristem ('promeristem') and the tissue immediately below, i.e. the 'primary meristem ring' or 'residual meristem', can be observed in certain seed plants seems beyond question. In the writer's view the relationship between the two tissues should be considered not only statically in terms of histology, as is so often the case in these investigations, but dynamically, having regard to the metabolic processes and interchanges which may be operative; moreover, these processes should be examined not only in the vicinity of the terminal meristem but as components of an integrated system in the formative region as a whole. Whether the hypothesis under consideration proves tenable for seed plants remains to be seen; some of the data presented by Helm, Kaplan, and other workers suggest that a positive finding may in fact be anticipated. Here it is appropriate to mention experimental investigations carried out by Jost (1891) on the development of vascular tissues in various woody plants. He came to the conclusion that the development of organs at the shoot apex is in many, though not in all cases, a *necessary condition for the differentiation of vascular tissue*.

In the hypothesis presented here the diffusion of the 'activating' substance (or substances) associated with the initial differentiation of vascular tissue is from the apical meristem backwards. In the development of the stele in lateral buds Esau has raised the question as to whether the vascular connexions develop from the lateral towards the main shoot or in the opposite direction, and further, if the direction is the same under all conditions. She observes that, if the concept of basipetal procambial differentiation of leaf-traces is

<sup>1</sup> The relevant literature has been fully reviewed by Esau.

applied to the problem of vascular development in axillary shoots, such shoots may be regarded as forming their connexions with the main axis by a downward differentiation of their leaf-traces. The writer's observations on bud development in *Matteuccia struthiopteris*, *Onoclea sensibilis*, *Dryopteris* spp., and other ferns afford definite indications, (i) of a basipetal initial differentiation of vascular tissue, and (ii) that in the development of the bud stele and its connexions with the shoot stele, bud leaf-traces may be in no way involved. A considerable body of evidence from dicotyledons, reviewed by Esau, supports the view that a basipetal *initial differentiation* of vascular tissue is general for both axillary and adventitious buds. This must be clearly distinguished from the *subsequent differentiation* where other processes are involved.

Avery (1940) has made reference to a number of investigations which suggest that substances such as indole-acetic acid may profoundly affect the transport of materials and may lead to the accumulation or mobilization of nitrogen and carbohydrates in proximity to the point of application. Now substances such as hetero-auxin probably owe their origin to the decomposition of proteins; for example, Thimann and Skoog (1934) have shown that whereas auxin is actively produced by growing buds none is produced by dormant buds. If taken together and applied in the present context these several observations suggest the existence of a close relationship between the maintenance of a terminal meristem in an actively formative condition, the production of activating substances during protein metabolism, the initial differentiation of vascular tissue, and the accelerated movement of nutrients to the formative region. The identity of the specific substance, if it is involved in the initial differentiation of vascular tissue, remains to be determined; it may be one of many by-products of protein metabolism.

## VII. SUMMARY

1. The classical investigations of the vascular system of pteridophytes have been almost entirely morphological in their inception and outlook. Criticisms of methods employed in these investigations relate to the neglect of studies of differentiation and development from the apex backwards at different stages in the ontogeny and to a tendency to consider the vascular system apart from the associated cortical tissues.

2. The need for an hypothesis to account for the *initial differentiation* of vascular tissues is discussed. The importance and implications of the apical meristem as an important seat of protein synthesis is briefly outlined and the following working hypothesis given: wherever the apical meristem of a shoot, bud, leaf, or root is in a state of active growth, of such a nature that the distinctive character of the meristematic cells is maintained, the initial differentiation of stelar tissue will be observable immediately below the apex and in the path of substances diffusing from it, one or more of these substances being causally involved in that process. What the diffusing substance (or substances) may be and how it operates is still a matter for conjecture.

3. Supporting evidence from different pteridophyte sources is given and the bearing of the hypothesis on investigations of the initial differentiation of vascular tissue in seed plants is discussed.

The writer has pleasure in acknowledging his indebtedness to Mr. E. Ashby for assistance in microscope preparations and photographic illustrations.

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#### DESCRIPTION OF PLATE III

Illustrating Professor C. W. Wardlaw's paper on Initial Differentiation of Vascular Tissue.

All figures are from untouched photographs.

FIG. 1. *Dryopteris aristata*. Longitudinal section (not quite median) through a shoot apex, showing the distinctive meristematic cells, *m*, the underlying vascular tissue, *v.t.*, in the initial phase of differentiation, and pith, *p*. The large superficial cell to the left is a leaf primordium. ( $\times 400$ .)

FIG. 2. *Dryopteris aristata*. Median longitudinal section through a bud, showing the dome-shaped region of vascular tissue in the initial phase of differentiation lying immediately below the apical meristem. ( $\times 37$ .)

FIG. 3. *Psilotum triquetrum*. Longitudinal median section through a rhizome apex, showing vascular tissue in the initial phase of differentiation lying immediately below the terminal meristem and also below a lateral meristem on left. ( $\times 100$ .)

FIG. 4. *Onoclea sensibilis*. Transverse section of a rhizome showing a bud experimentally induced. This has developed from a detached meristem. The bud is seen in median longitudinal section and its vascular system extends from the terminal meristem backwards and ends in the outer cortex of the parent rhizome. ( $\times 60$ .)

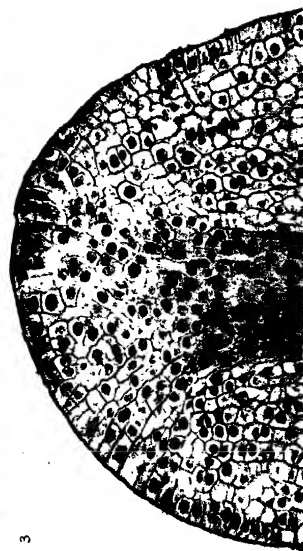
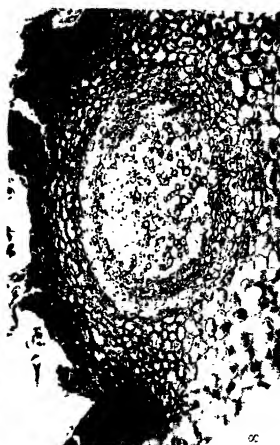
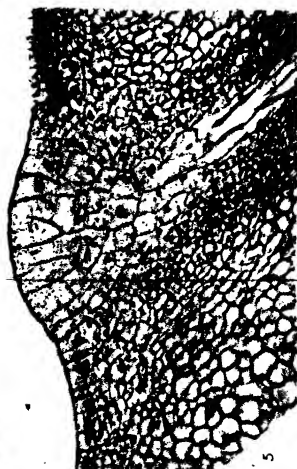
FIG. 5. *Selaginella Willdenowii*. Median longitudinal section through an angle meristem (i.e. through a young rhizophore apex). The vascular tissue is coextensive in diameter with the meristem and originates immediately below it. ( $\times 225$ .)

FIG. 6. *Matteuccia struthiopteris*. Longitudinal radial section of a rhizome, showing a detached meristem in which growth has been induced. On the right a parenchymatous development has taken place and there is no indication of differentiation of vascular tissue: on the left an active apical meristem has become organized and behind it can be seen a marked development of vascular tissue. ( $\times 60$ .)

FIG. 7. *Hymenophyllum demissum*. Longitudinal median section of a rhizome apex, showing the initial differentiation of vascular tissue immediately below the apical meristem. A leaf primordium, not seen in this section, is present to the left of the rhizome apex.

FIGS. 8 and 9. *Dryopteris filix-mas*. Transverse sections of an erect shoot, showing, in Fig. 8, the stele of an experimentally-induced bud in the outer cortex. Lower down, in Fig. 9, the same stele ends blindly, i.e. without making connexion either with the shoot stele or with a leaf-trace. ( $\times 60$ .)



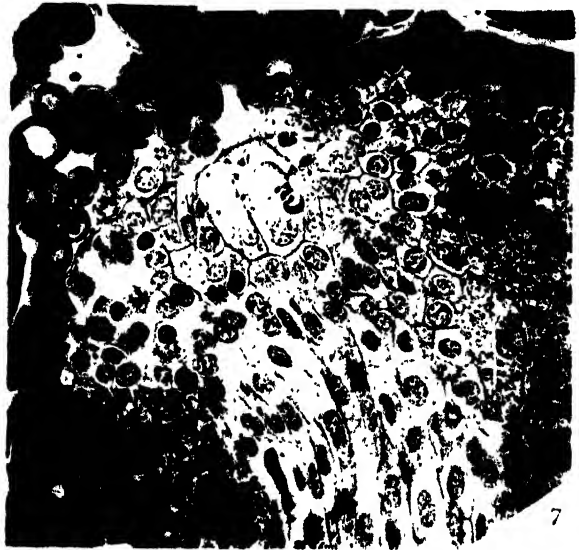


WARDLAW - DIFFERENTIATION OF VASCULAR TISSUE.

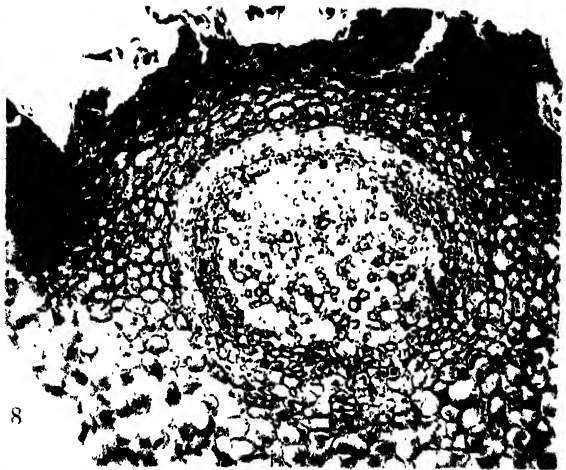
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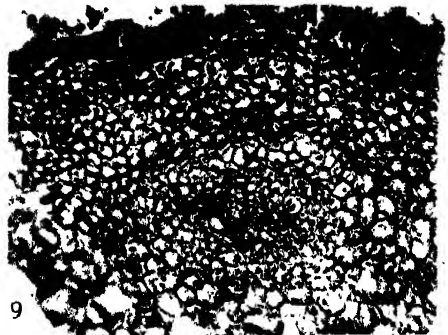
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# Acid Metabolism and Respiration in Succulent Compositae

## II. Respiration during Starvation in *Kleinia radicans*<sup>1</sup>

BY

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With sixteen Figures in the Text

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### INTRODUCTION

IN the experiments described in Part I of these studies (Thoday and Jones, 1939), with succulent stems of *Kleinia articulata*, the course of respiration during starvation did not show some of the features which characterize Cherry Laurel and other non-succulent leaves, nor were diurnal fluctuations of respiration rate observed like those found by Bennet-Clark in his experiments with leaves of Crassulaceae, although disappearance of malic acid and diurnal fluctuations of acidity had been demonstrated. Lack of homogeneity in the material, however, made doubtful the interpretation of these results. Accompanying the differentiation of tissues in the stem of this plant there are marked differences in metabolism between different zones on which the investigation was designed to throw light. Moreover the different parts of one stem segment were not of the same age or stage of development; for example, the storage of calcium and malate in the pith increased progressively from apex to base.

Both kinds of heterogeneity contribute to a slurring over of any changes that may occur. Even in a relatively uniform foliage leaf, changes in the

<sup>1</sup> This paper embodies part of a thesis presented by the second author for the degree of Ph.D. in the University of Wales.

observed respiration rates spreading over many days represent the statistical effect of changes in individual cells, which, even when they exactly correspond in nature, do not coincide in time. In each cell they must be of much shorter duration, and therefore more striking could they be measured in isolation. Some might represent even rapid drastic changes in the course of metabolism. With our more complex material the obscuring of effects might well be further accentuated.

The leaves of *Kleinia radicans* (Jackson and Thoday, 1939) provide, from this point of view, a simplified version of the stem of *K. articulata*. On the one hand they are differentiated into a superficial chlorenchyma surrounding an extensive colourless water tissue in which calcium malate abounds. On the other, the whole leaf may be regarded as approximately of the same age. It appeared therefore that the behaviour of tissues similar to those in the stem of *K. articulata* might in this leaf be exhibited more sharply and characteristically, though the results would still combine effects due to contrasting tissues.

## RESULTS

### Acidity

Preliminary qualitative tests showed that malic acid was present and no other acid was detected. Quantitative determinations at different times of day, using successive leaves from the middle region of the prostrate shoot, confirmed the characteristic diurnal fluctuation, the total malic acid falling during the day and rising again during the night. The following experiments (Table I) illustrate this. The fresh weight of each leaf is given in grammes and the malic acid as milligram equivalents per 100 gm. The methods used here have been given earlier (Thoday and Jones, 1939).

TABLE I

Expt. 72. May 22-3, 1936.

|                    | Leaf wt.  | Acid. |   |
|--------------------|-----------|-------|---|
| May 22: 10.15 a.m. | 1.676 gm. | 11.3  | Bright morning, afternoon<br>dull, drizzle. |
| 3.45 p.m.          | 1.466     | 9.8   |   |
| 9.15 p.m.          | 1.378     | 10.9  |   |
| May 23: 9.15 a.m.  | 1.660     | 13.0  |   |

Expt. 73. May 26-7, 1936.

|                   |       |      |                          |
|-------------------|-------|------|--------------------------|
| May 26: 8.45 a.m. | 1.730 | 13.9 | Sunny.                   |
| 12.15 p.m.        | 1.460 | 11.0 |                          |
| 3.45 p.m.         | 1.764 | 10.6 | Sun lower, getting dark. |
| 7.15 p.m.         | 1.623 | 9.4  |                          |
| 10.15 p.m.        | 1.750 | 9.5  |                          |
| May 27: 9.0 a.m.  | 1.603 | 12.3 |                          |

Such results are a good indication of the consistency of the analytical technique and the close agreement between successive leaves on a stem.

In a later experiment (Table II) younger leaves were used and it was necessary to take three leaves for each determination. The titratable acidity

was determined, as well as the total malate. The shoots were in darkness from 7.30 p.m. to 8.30 a.m.

TABLE II

Expt. 74. Sept. 22-3, 1936.

|                     | Malic acid.      | Titrate acidity.                |
|---------------------|------------------|---------------------------------|
| Sept. 22: 7.30 p.m. | 10.9 mg. equivs. | (Just alk. to phenol-phthalein) |
| Sept. 23: 8.30 a.m. | 29.3 "           | 3.3 mg. equivs.                 |
| 7.30 p.m.           | 11.4 "           | 0.2 "                           |

It will be seen that the acid content is comparable with that in the stem of *K. articulata* and is much higher than that in leaves of the latter.

In view of the results obtained by Bennet-Clark (1933, pp. 148, 154) with leaves of Crassulaceae, an attempt was made to determine the changes of acidity in prolonged darkness. Several shoots were kept in the dark at room temperature (about 18° C.). Three leaves were taken on each occasion for the determination of acidity, so as to obtain some indication of the variability of the material, each leaf being analysed separately, and its position on the shoot recorded.

The data indicated the usual rise in acidity during the first night period, but thereafter a maintenance at the high level for five days. Sampling errors were of sufficient magnitude to obscure small changes and to necessitate the use of more material than our supply permitted if minor fluctuations were to be detected. Shortage of material also precluded further investigation of the acid content at different temperatures.

#### *Carbon dioxide output during starvation.*

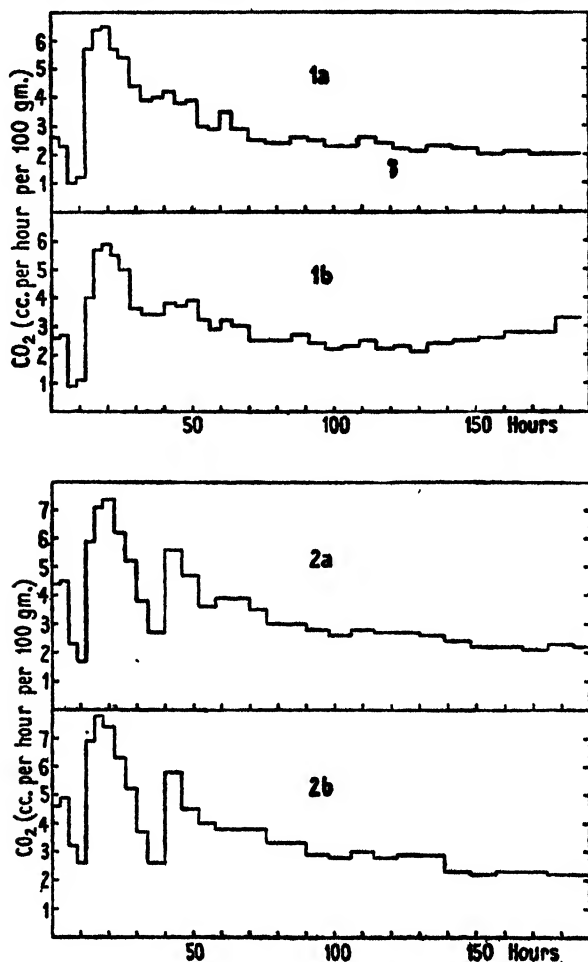
Single leaves were used for these experiments, and in general two identical experiments were run in parallel. Comparison of the results of such pairs of experiments with adjacent leaves from the same shoot gave ample proof of the reliability of the technique and of the close similarity of behaviour of adjacent leaves. As will be seen later, the series of experiments, taken as a whole, presents a consistent picture.

The leaves weighed from about 0.7 to 1.7 gm., mostly about 1 gm. or a little more. The cut ends were sealed with wax immediately after detachment from the plant. The temperature was maintained throughout at 25° C. in the thermostat already described (Thoday and Jones, 1939, p. 679). As a rule the experiments were started when daylight was failing, not later than 8 p.m.

The two graphs of CO<sub>2</sub> output given in Fig. 1, for adjacent leaves from one of the shoots used for the acidity estimations, agree in showing a fall in rate to a low level during the first 12 hours, followed by a rapid rise to a peak about 6 times the minimum rate. The fall from the peak value occurred about 24 hours after the initial fall and there is some indication of a second rise, though on this point these two experiments by themselves would be inconclusive. The general trend of the graphs continues downwards with diminishing rapidity. In expt. 80 a minimum was reached after the fifth 24-hour

period, after which the rate rose. In expt. 79 the rate continued to fall throughout the 8 days for which the two experiments were continued.

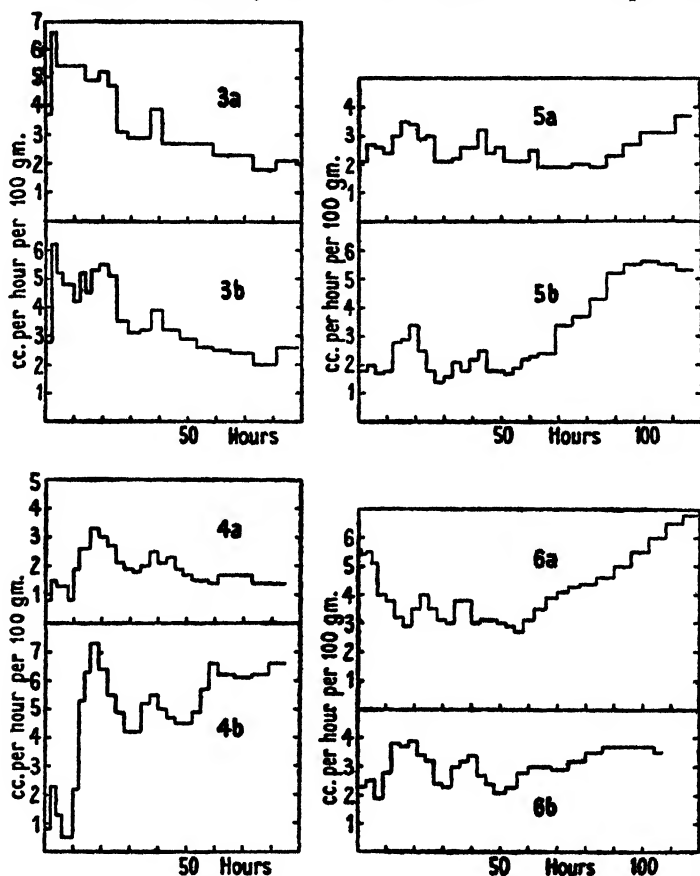
The results for the next pair of experiments are given in Fig. 22. The two graphs are not only closely similar but they are strikingly parallel with that



FIGS. 1 and 2. *Kleinia radicans*: Figs. 1a and 1b, expts. 79, 80, July 9-17, 1936; started 8 p.m. Adjacent leaves (numbered from the tip of the shoot): a, leaf 13, 1.00 gm.; b, leaf 12, 1.03 gm. Figs. 2a and 2b, expts. 81, 82, July 20-30, 1936; started 8 p.m. Adjacent leaves: a, leaf 13, 0.735 gm.; b, leaf 12, 0.683 gm.

given by Bennet-Clark for detached leaves of *Crassula lactea*, at 21° C., the only difference of note being the very low level of the first minimum in the latter. This time the second peak is made unmistakably clear by the second minimum which precedes it. In both these experiments the CO<sub>2</sub> output continues to fall right to the end of the period of experiment, in this case 10 days, neither graph giving any sign of the rise shown in expt. 80.

The two pairs of experiments, carried out during July, while they showed that adjacent leaves on the same shoot give closely similar results, differed from one another sufficiently to raise the question of the uniformity of the material available; how closely would the behaviour of corresponding leaves



FIGS. 3-6. *K. radicans*: Figs. 3a and 3b, expts. 83, 84, Sept. 7-11, 1936; started 8 p.m. Leaves from different shoots: a, 0.918 gm.; b, 0.770 gm.: Figs. 4a and 4b, expts. 85, 86, Sept. 11-15, 1936; started 8 p.m. Leaves from different shoots: a, 1.561 gm.; b, 0.972 gm.: Figs. 5a and 5b, expts. 87, 88, Sept. 15-20, 1936; started 8 p.m. Leaves from different shoots: a, 1.210 gm.; b, 1.350 gm. Figs. 6a and 6b, expts. 89, 90, Sept. 20-7, 1936; started 8 p.m. Leaves from different shoots: a, 1.550 gm.; b, 2.130 gm.

on two different shoots agree if taken at the same time? When the experiments were resumed in September attention was first directed to this point.

Figures 3 and 4 present the results of two pairs of experiments (Nos. 83-4, 85-6) for each of which corresponding leaves from different shoots were taken. Each pair was continued for about four days, from September 7 to 11 and 11 to 15 respectively. They thus cover the period of marked rhythmical fluctuations in expts. 81-2. Here the differences are considerable. While there are indications of the rhythm in all four graphs, the general impression

is one of irregularity. In the one experiment, 86, in which a regular diurnal rhythm is clear, the  $\text{CO}_2$  output remains at a much higher general level, instead of falling as in the July experiments.

In the next four experiments (Nos. 87–90; Figs. 5 and 6), carried out similarly in the latter half of September, the rhythm can still be seen, but an early general rise is evident in all and is conspicuous in expts. 88 and 89.

Experiments 91 and 92, begun at the end of October, showed further

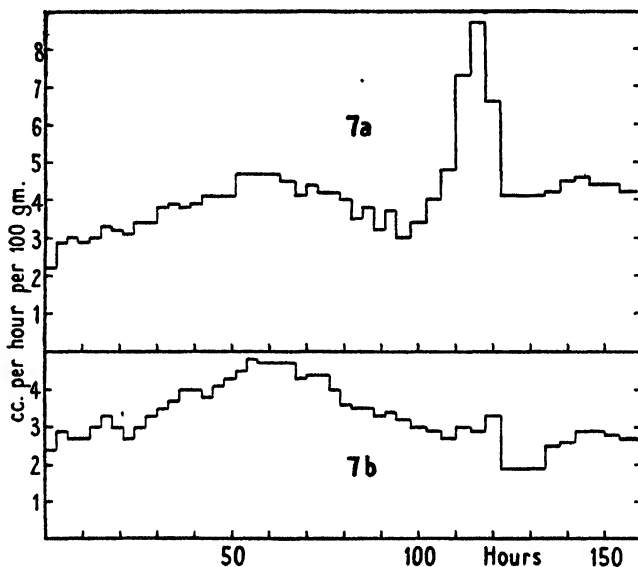
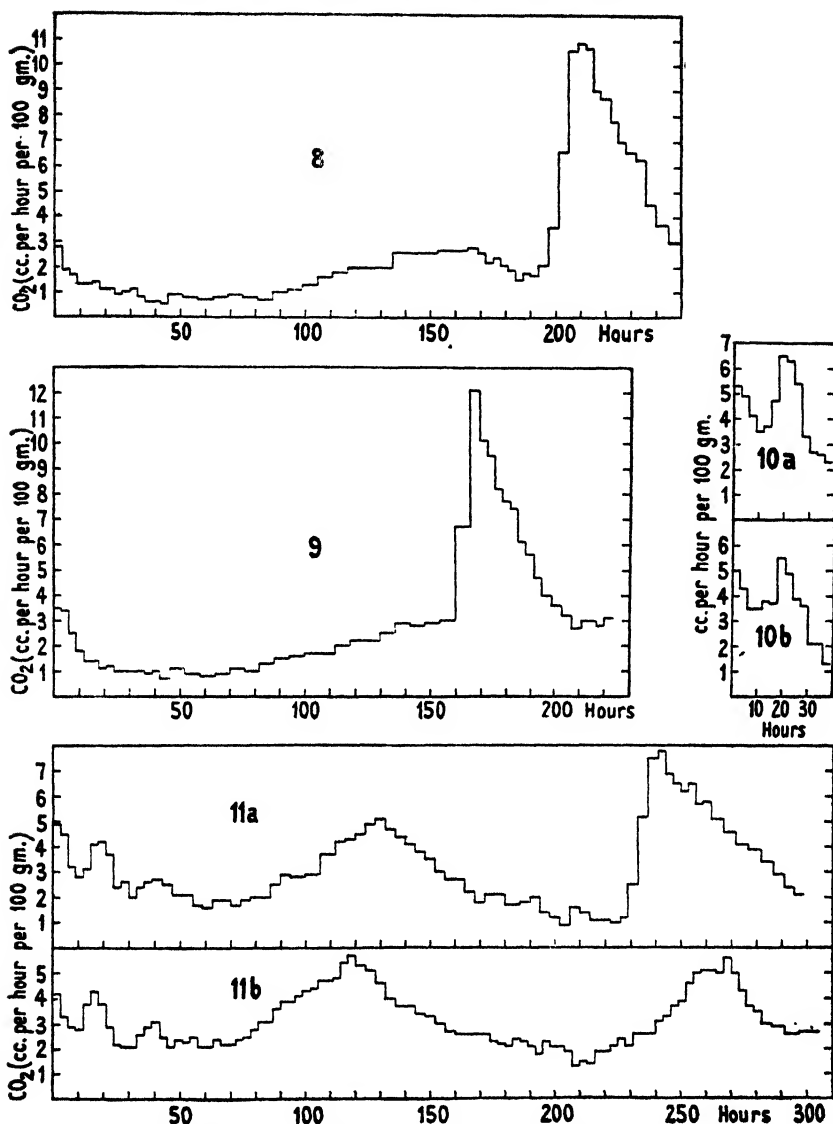


FIG. 7. *K. radicans*: expts. 91, 92, Oct. 29–Nov. 5, 1936; started 6 p.m.  
Fig. 7a, leaf, 1.470 gm.; Fig. 7b, leaf, 1.708 gm.

differences. There is no evidence in these graphs (Fig. 7) of the characteristics of the earlier part of the July graphs. The  $\text{CO}_2$  output rises from the beginning, with irregularities of doubtful significance, and after about 60 hours falls again. In expt. 91 a new phenomenon appears: after about 100 hours (4 days) the  $\text{CO}_2$  output begins to rise rapidly and reaches a high peak value, falling again as rapidly to a value comparable with that reached in the first rise. This burst of  $\text{CO}_2$  production is apparently completed within 24 hours. When this leaf was examined at the end of the experiment it was dark green and injected with sap. All the leaves previously used had appeared quite healthy at the end of their period of starvation in darkness.

By this time it was clear that what had appeared at first sight to be irregular variations in behaviour actually fell into a series, representing a sequence of changes connected with the advancement of the season. The next experiments were designed to determine the operative causes. The leaves were taken from a fresh batch of plants grown from cuttings made the previous September. By November they were comparable in vigour and general appearance with the shoots used in July. (All the experiments so far described, from July onwards, had been with leaves from the one batch of plants.)



FIGS. 8-11. *K. radicans*: Fig. 8, expt. 93, Nov. 12-22; started 5 p.m. Leaf, 1.494 gm. (From fresh batch of plants, started as cuttings in Sept.) Fig. 9, expt. 94, Nov. 12-21; started 5 p.m.; leaf, 1.380 gm. Figs. 10a and 10b, expts. 95, 96, Nov. 24-5, after 5 days' light treatment; started 3.30 p.m.; leaf a, 0.847 gm.; b, 0.830 gm. Fig. 11a, expt. 99, Nov. 27-Dec. 10, after 8 days' light treatment; leaf, 0.925 gm. Fig. 11b, expt. 100, Jan. 1-14, after 17 days' light treatment; leaf b, 1.012 gm. Both started at 5 p.m.

The experiments using the new batch were continued longer than previously in order to see whether the burst of CO<sub>2</sub> shown in expt. 91 is a general feature when starvation is pushed far enough. Expts. 93 and 94 (Figs. 8 and 9) both show this feature, though they differ in its incidence. In the former, CO<sub>2</sub>



production was more conservative; the initial fall is followed by a rise to the seventh day, after which the output falls again before the outburst begins at about 200 hours. In expt. 94 the output is at a higher level throughout. The level is still rising when the outburst supervenes after only 160 hours. This, however, is some 60 hours later than in expt. 91. (The pH of the sap from the injected leaves was respectively 8.5 and 8.9.)

Age and vigour thus affect the rate of onset of the different phases of the starvation sequence; but the initial phase of the earlier experiments is still absent. The main differences in external conditions which the progress of the season brings are a diminution in the duration, and also, especially in the cloudy climate of Bangor, in the intensity, of the illumination. The average temperature was also lower, although the succulent house was maintained at a minimum temperature of 45 to 50° F. by thermostatically controlled electric heating.

A box of the September cuttings was therefore brought from the greenhouse and put in a chamber, lighted from above by a 500-watt lamp through a running-water screen that formed the roof of the chamber. A time switch was adjusted to provide alternating periods, 12 hours light and 12 hours dark. The temperature varied from 18° to 22° C. when the light was on, falling to about 15° C. in the dark intervals.

Experiments 95 and 96 were started when the shoots had been 5 days in the light chamber. The graphs (Fig. 10) show unmistakably the features of the late summer experiments, an initial fall followed by a rise to a peak value. These two experiments were hardly continued long enough to show for certain whether or not the output would have risen again, to a second peak.

The two following experiments (Fig. 11) were continued long enough to obtain a complete record of the whole sequence of events from the first phase of diurnal fluctuations to the final outburst of CO<sub>2</sub>, with injection of the tissues. Expt. 99 was started after 8 days in the light chamber, Expt. 100 after 17 days. In view of the differences in duration of light treatment and in date, the degree of parallelism between the two graphs is striking.

Dealing first with the initial phase, both graphs show, like those for previous experiments, an initial fall followed by a rise to a peak, in neither case, however, quite so pronounced as before; but both show also a second peak about 24 hours later. As regards this initial phase, the graphs for all four experiments are most nearly comparable with those previously obtained in July.

This first phase lasted about 48 hours. After an interval of another 24 to 36 hours the CO<sub>2</sub> output begins to rise again. This time the rise continues steadily for 48 hours, reaching a peak value in excess of the previous peak values, and is followed by a fall of longer duration to a lower minimum rate. This feature has already appeared in expts. 91 and 92 and is partially represented in expts. 88 and 89. Finally, in expt. 99, is seen the outburst of CO<sub>2</sub> of expts. 91, 93, and 94. In expt. 100 there is also an enhanced output of CO<sub>2</sub> but it is not so sharply defined.

Expt. 99 provides a clear picture of the three phases of starvation as it affects the  $\text{CO}_2$  output, each distinct from the others. In the previous experiments one or other is lacking or less distinctly shown, and in some there is more or less overlapping of successive phases.

Of the three phases, the first finds a parallel in the results obtained by Bennet-Clark with the leaves of Crassulaceae. The second phase may be

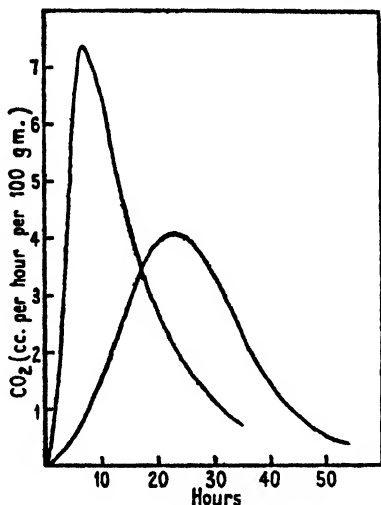


FIG. 12. For explanation, see text.

compared with the hump that characterizes the  $\text{CO}_2$  output graphs of starving Cherry Laurel and other similar leaves, although it is not here associated with yellowing of the chlorenchyma.

For the third phase, on the other hand, there appears to be no previous parallel. The peak value is reached in a remarkably short space of time—in expt. 94 in less than 10 hours from the onset of this phase. It is, moreover, the highest. In a December experiment (expt. 103, see below, p. 201) it reached nearly 16 c.c.  $\text{CO}_2$  per hour per 100 gm., in others 8–14 c.c.; as compared with less than 8 c.c., the highest peak in phase (1) (expt. 82); 3–5 c.c. in phase (2); the minimum before and after phase (2) varying between 1 and 3 c.c.

The shape and varying duration of this third phase suggested a drastic change, occurring in all the cells of a tissue in more or less rapid succession, such as a sudden mobilization of a respiratory substrate, the concentration of which then diminishes according to the law of mass action. Curves have been constructed on the assumptions (1) that the concentration in any cell is halved in 8 hours, (2) that the onset of the change in different cells follows a normal probability distribution, the standard deviation of the times of initiation about the mean time being taken as 2 hours and 8 hours respectively. It will be seen (Fig. 12) that the first of these curves reproduces fairly closely the characteristics of the relevant part of Fig. 16 and that the other illustrates the greater spread shown in Figs. 15 and 18 in various degrees.

A point to be noticed is that when phases overlap they often appear to be superposed, the  $\text{CO}_2$  production being additive. How far a release of supplementary substrates in the same cells might account for this, or whether it represents coincidence of changes in different tissues, must remain for further work to elucidate.

### *Oxygen intake.*

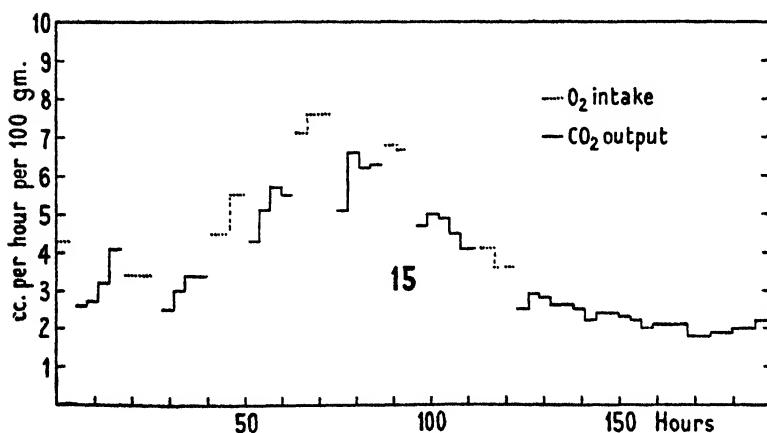
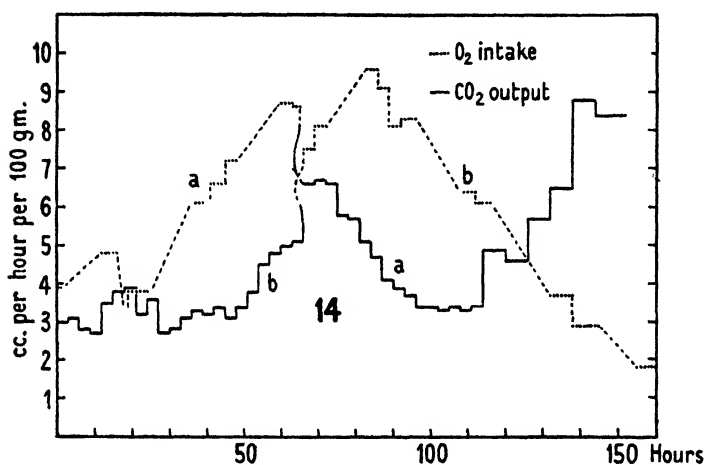
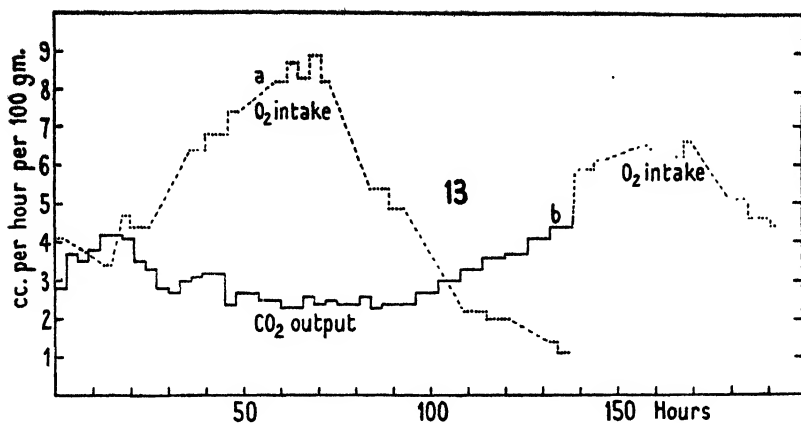
In attempting to obtain light on the nature of the changes expressed in the  $\text{CO}_2$  output curve, parallel investigation of the  $\text{O}_2$  intake was undertaken. Unexpected difficulties were encountered which prevented the accurate estimation of the  $\text{CO}_2/\text{O}_2$  ratio, though evidence was obtained that oxidative processes are involved throughout.

When a leaf was enclosed in the manometric respirometer used for measurement of oxygen intake the form of the respiration curve obtained did not run parallel to the curve obtained for  $\text{CO}_2$  output with an adjacent leaf in the other apparatus. In view of the observations of Bennet-Clark (1932) on the oxygen intake of leaves of *Sedum prealtum*, a difference between the two curves was not unexpected; but it soon became clear that the discrepancy was different in kind. The oxygen curve (Fig. 13*a*) invariably began after a few hours to rise, reaching a high maximum level in 2 or 3 days, then falling to a low level after about 5 days, from which it never recovered. At room temperature ( $18^\circ \text{C.}$ ) the rise was somewhat delayed but the course of the curve was otherwise very similar. Evidently the conditions in the manometric apparatus in some way affected the leaves: from their injected condition after a few days it was inferred that the influence was detrimental.

Some attempt was made to discover the effective factor, though circumstances prevented the continuance of this part of the investigation, and it has not yet been possible to take it up again. The most obvious difference between the conditions in the manometric and the other apparatus was that in the latter a slow current of air was passing continuously. This air current was, moreover, nearly saturated by passage through a weak solution of baryta.

In the closed manometric apparatus the air was in contact with 10 per cent. KOH, and therefore drier. The possible influence of this difference of humidity was tested by substituting 10 per cent. KOH for the baryta in the air current. This did not alter in any way the form of the carbon dioxide output curve.

Accumulation of emanating gases such as ethylene seemed a possibility, by analogy with acid fruits. When the oxygen intake was determined intermittently, the apparatus being closed for as short a period as practicable and left open between the observations, the curve was somewhat extended but not altered in character. Nor was there any significant change when, with a specially modified apparatus, an air current was drawn through the respiration chamber during the intervals. Breakdown, indicated by injection of the air spaces with sap, was delayed but not prevented.



FIGS. 13-15. *K. radicans*. Fig. 13, expts. 114 and 115; started Sept. 29, 1936, 8 p.m. Three leaves for each expt.: a, 2.767 gm.; b, 1.855 gm. Fig. 14, expts. 112, 113; started Sept. 28, 1936, 7 p.m. Three leaves for each expt.: a, 2.164 gm.; b, 2.464 gm. Fig. 15, expt. 116; started Oct. 6, 1936, 4 p.m. Three leaves, 2.630 gm.

The introduction of a small tube of sulphuric acid as an absorbent for ethylene did not alter the curve of  $O_2$  intake; it is possible that the surface of sulphuric acid exposed might have been inadequate. Introduction of carbon granules along with the leaf was likewise ineffective. When, however, finely divided animal charcoal covered the bottom of the flask, replacing the potash normally used to absorb  $CO_2$ , injection of the leaves was delayed; but the lateness of the season prevented further investigation of this avenue.

Meanwhile experiments had been carried out in which leaves were transferred from one apparatus to the other at various times. These showed that the course of  $O_2$  intake corresponded fairly closely with  $CO_2$  output. The  $CO_2/O_2$  ratio could not be satisfactorily estimated, but was less than unity throughout.

The following examples will suffice to illustrate the phenomena and justify the inference that oxidative reactions are involved at all stages.

Figure 13 shows the characteristic form of the  $O_2$  intake curve in our experiments and the quite different course of the  $CO_2$  output from similar leaves in the other apparatus. On the sixth day the latter were transferred to a manometric apparatus and their  $O_2$  intake followed. The  $CO_2$  output had already begun to rise (phase 2). The  $O_2$  intake curve continues the rise at a rather higher level before falling.

Figure 14*a* shows again the  $O_2$  intake rising to a peak. Transference to the  $CO_2$  apparatus shows the  $CO_2$  output falling parallel with the expected course of the  $O_2$  intake at a rather lower level. In Fig. 14*b*, the  $CO_2$  intake curve is first recorded till it is rising in the second phase. The  $O_2$  intake continues the rise, at a higher level, before falling.

The rise in  $CO_2$  intake shown in Fig. 14*a* after 5 days is interesting: it suggests that the third phase may still occur if enclosure has not been too prolonged. This phase is suppressed by enclosure, which on the other hand appears to accelerate phase 2.

In the experiment illustrated by Fig. 15 the transference from one apparatus to the other was repeated a number of times. The results show the  $CO_2$  output and  $O_2$  intake rising and falling on the whole together, with some irregularity in the initial period.

Finally Fig. 16*a* shows the result of transferring a leaf from the continuous current apparatus at the peak of phase 3. The  $O_2$  intake closely parallels the expected course of the  $CO_2$  intake, which is illustrated for a second leaf, *b*. It is a point of interest in these two experiments, carried out in December, that the third phase set in very early, after about 3 or 4 days respectively, as compared with 7 or 8 days in mid-November and 9 or 10 days after bright illumination. With the old batch of material, at the end of October, it appeared after 4 days. It may also be significant that the peak values in these December experiments were the highest observed.

#### *Experiments with leaves of other species of Kleinia.*

A few experiments have been carried out with leaves of other species of

Kleinia. They show that *K. aizoides* and *K. ficoides* are also intolerant of enclosure. With *K. repens*, on the other hand, the  $O_2$  intake and  $CO_2$  output curves did not differ in form and there was no evidence of injury from enclosure. It is perhaps of significance in this connexion that the content of

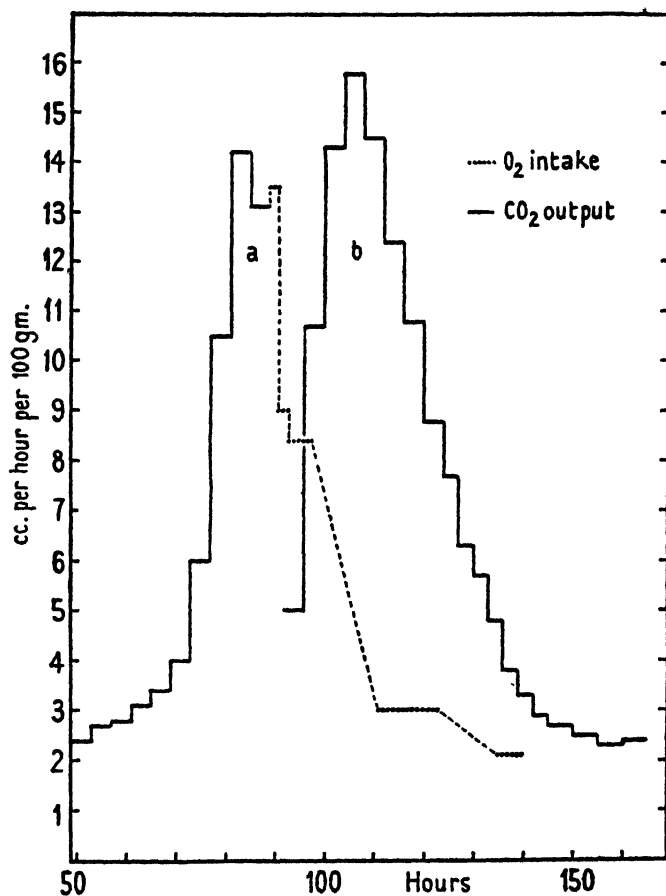


FIG. 16. *K. radicans*. Expt. 103; started Dec. 2, 1936, 5 p.m.  
Leaf a, 1.515 gm.; b, 1.316 gm.

both malic acid and calcium are much lower in this species than in the other three.

Whether any of these species exhibit the third phase of  $CO_2$  output has not yet been determined. It is clear, however, that they are more resistant to starvation, as experiments were continued for 10 days in late October with *K. aizoides*, for 9 days in December with *K. ficoides*, and for 10 days in January with *K. repens*. The peak of phase 2 was reached in about 7 days by *K. ficoides*; but no clear indication even of the onset of this phase was observed with the other two species within the periods specified.

## GENERAL REMARKS

The results of the experiments with *Kleinia radicans* have fully realized the expectation that relatively uniform material would allow any distinct phases in the respiration sequence to be more readily distinguished and assessed in relation to the behaviour of the individual cells and tissues which contribute to the observed statistical resultant data. There is still some overlapping of phases, but they are clearly distinguishable in the most favourable cases.

Looking back to the results obtained with *Kleinia articulata* (Thoday and Jones, 1939) it will be seen that further work is needed to discover whether the first phase of diurnal fluctuation of  $\text{CO}_2$  output would be exhibited by the stem of that plant under appropriate conditions, with reference particularly to illumination and stage of development.

The third phase in leaves of *K. radicans* would adequately account for the rise of respiration in the stem of *K. articulata* when the pith begins to show injection. The pith is relatively more bulky than the water tissue of the leaf in proportion to its chlorenchyma, and the onset of injection more gradual. In both, the characteristic rise of pH above 8 is associated with injection.

The second phase, however, is not apparent. It is in keeping with the picture already developed to suppose that injection, due to exhaustion of the pith, supervenes before the reserves of the outer tissues have been depleted to such an extent as to bring on phase two. Evidently further work is necessary before the situation can be resolved with any degree of certainty, but the results obtained with *K. radicans* make the attempt appear promising.

## SUMMARY

In the leaves of *Kleinia radicans* a high malic acid content was found by analysis, and the normal diurnal fluctuation of acid content was demonstrated. In prolonged darkness, the acid content remained for some days at the high level reached during the first night.

The  $\text{CO}_2$  output from single leaves in prolonged darkness was followed, with the micrometric apparatus previously described. The graphs obtained varied in form with the season and the vigour of the plants. In summer the leaves endured longer than in winter before collapsing, and for 1 or 2 days showed diurnal fluctuations in  $\text{CO}_2$  output very similar to those found by Bennet-Clark for leaves of *Crassula lactea*. As the season advanced this initial phase (1) disappeared, and two later phases set in progressively earlier. These were (2) a rise followed by a fall, occupying about 4 days; and (3) a striking rise, mostly very rapid, to a much higher peak than in previous phases, followed immediately by a rather less rapid fall. Phase 3 coincided with injection of the tissues with sap. The curves fit fairly well what might be expected if breakdown made a supplementary substrate for  $\text{CO}_2$  production suddenly available in individual cells, and progressed rapidly through the tissues.

In some instances, phases 1 and 2, or 2 and 3, were telescoped together, more or less, the CO<sub>2</sub> output appropriate to 1 or 3 being apparently superposed on that appropriate to 2.

Healthy winter plants were brought into a condition approximating closely to that of summer plants by the higher light intensity of a 500-watt Osram lamp, given for 12 hours daily for several days. They then showed both the first phase of diurnal fluctuation and the whole sequence fully extended.

Enclosure in stagnant air altered the course of respiration drastically. The oxygen intake soon began to rise, reached a maximum in 2 or 3 days, then fell again to a low level: the leaves were then found to be prematurely injected.

Alternate determination of O<sub>2</sub> intake and CO<sub>2</sub> output for the same leaf showed that the two were running parallel in this abnormal course, and also at different parts of the normal CO<sub>2</sub> output graph of leaves in a current of air. The data are, however, inadequate to determine the relations between CO<sub>2</sub> production and O<sub>2</sub> absorption during the primary phase of diurnal fluctuation.

Some preliminary evidence was obtained that in absence of an air current the leaves were being poisoned by volatile products of their own metabolism: alternation of enclosure with periods of free diffusion or circulation of air slowed down the rise and fall, and the provision of a large surface of finely divided carbon deferred injection of leaves enclosed with it.

Leaves of *K. aizoides* and *K. ficoides* were found to be affected by enclosure like those of *K. radicans*. With those of *K. repens*, on the other hand, the CO<sub>2</sub> output and O<sub>2</sub> intake graphs were similar in form. The last species differs from the other three in having a much smaller, very variable calcium content and much less malate.

#### ADDENDUM

In a paper that has come to hand since the above was written Bennet-Clark and Bexon (1943) show that when beetroot tissue slices are transferred from water to juice expressed from the same root the rate of respiration may be doubled, and trace the effect in large part to malic and other organic acids present in the juice. It seems highly probable that phase 3 in *K. radicans* is an example of this phenomenon in the intact plant organ.

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# The Growth in Thickness of *Oreodoxa regia* H. B. and K

BY

T. PETCH

With two Figures in the Text

THE growth in thickness of the stems of palm trees in the tropics has been the subject of several papers, but periodic measurements have not been numerous, and the writers have not remained long enough in the tropics to furnish any details of its progress and duration.

Möller measured the girth of a specimen of *Euterpe oleracea* in Brazil at breast height, 1.5 metres below the lowest leaf. The clear stem would thus be about 3 m. high, and the point of measurement at about half its height. His results, as cited by Kranzlin (1906), show an increase in girth from 24.5 cm. to 29 cm. in 21 months.

Kraus (1911) measured palm stems at Buitenzorg, Java, during a period of 3 months, November 1893 to February 1894, which he stated was the period of most active growth at that station. From his observations he concluded that in 2 months young palm stems show an increase in thickness over their whole length from apex to base, large enough to exclude all doubt. Some of the increments recorded are so small that they appear to be within the limits of error, but a specimen of *Rhopaloblaste hexandra* Schaeff., with a clear stem 1.46 m. high, increased in girth from 80 to 82.7 cm. (3.4%) at the roots, from 55.4 to 59.5 cm. (7.4%) at 56 cm. from the base, from 47.5 to 51.3 (8.1%) at 96 cm., and from 46 cm. to 47.9 (4.1%) at the lowest leaf sheath. Similarly, a *Drymophloeus singaporensis* Hk., 1.75 m. high, increased by 4.3 cm. (3.8%) at the base, and 5.5 cm. (11.5%) at the apex. The latter is a greater percentage increase than any recorded by Kraus for *Oreodoxa regia*.

Schoute (1912), who visited Buitenzorg in 1903, considered that he could overcome the necessity for a prolonged residence in the tropics by the following method. He measured the girth of several examples of the same species at the same height from the ground and also the length of the bare stem. If the taller trees showed a greater circumference at the given height, he concluded that that species increased in girth. Practically he assumed a correlation between height and girth. The falsity of his method is illustrated by the following examples. He measured three specimens of *Acanthophoenix rubra* Wendl., and concluded that his data indicated that a good growth in thickness was very probable. On the other hand, he measured seven examples of *Cocos nucifera* L., and concluded that growth in thickness in that species could be only insignificant. If, however, he had measured only three examples

of the latter (his first, second, and sixth) he must have come to the same conclusion as in the case of *A. rubra*.

What may be termed the 'primary thickening' of a palm stem is similar to that which occurs in a grass. For some time, sometimes for several years, only the leaves of the seedling palm can be seen above ground. During that period the bud increases in diameter, and the successive leaf-scars lie on an externally convex surface, shallow in the case of large species. As growth continues, the margin of this surface becomes more and more vertical, longer internodes are produced, and, as the leaves fall, the stem becomes visible. The increase in the diameter of the bud, and the consequent outward curvature of the surface of the stem, may continue above ground, but in general this increase soon ceases and the diameter of the bud begins to diminish. Thus a swelling, or bulb, is formed at the base of the stem, sometimes insignificant, sometimes pear-shaped and, in some examples of *Cocos nucifera*, up to 1 m. high and 70 cm. diameter. Above the swelling the stem is usually cylindrical, tapering slightly upwards. A well-grown coco-nut palm at Peradeniya measured 24 cm. in diameter at 1 m., and 20.5 cm. at 5 m. from the ground. There is no obvious secondary thickening in the coco-nut palm.

In the Kitul palm (*Caryota urens* L.) the stem of a fully-grown example is gradually thicker upwards. A felled specimen measured 115 cm. in girth at 2.4 m., and 135 cm. at 6.25 m. at the base of the lowest leaf. This is probably due to continued increase in the diameter of the bud. 'Secondary thickening', i.e. increase in diameter of the stem after it has been formed or become visible, is indicated in the Talipot palm (*Corypha umbraculifera* L.) by the triangular fissures in the old leaf bases which usually persist at the base of the stem. (The lower leaves of the Talipot are usually cut off, not allowed to fall naturally.) It is, however, a well-known phenomenon in the Royal palm (*Oreodoxa regia*), which also bears on old trees an obvious swelling, the barrel, on the upper part of the stem; and in the hope of obtaining some information on these developments the following observations were undertaken.

In May 1915 a row of three *O. regia* and three *O. oleracea* seedlings was planted in my garden at Peradeniya, Ceylon. Of these, only one *O. regia* remained when I returned from leave in May 1916. Its subsequent growth was slow, and its stem was not visible above ground until November 1919. Peradeniya (1,600 ft.) is near the upper limit for many tropical species in Ceylon. In the following account, the leaf which became detached on November 11, 1919, is called the first leaf; it is not of course the first, but the first to leave a leaf-scar or ring, visible above ground. Measurements of girth were made in inches to the nearest eighth of an inch along the upper edge of each leaf-scar, and also at the base of the leaf-sheath above. Only the former measurements are used in the following summaries, and they have been converted into centimetres for convenience of publication. These measurements were repeated weekly, at about 9 a.m., at each ring until I left Ceylon in May 1925. On my return a few measurements were made in 1926,

but as I was then stationed fifty miles away continuous records were not possible. A last set of measurements was made in 1928, the year in which I finally left Ceylon, but the bungalow had then ceased to be occupied by



FIG. 1.

FIG. 2.

FIGS. 1 and 2. *Oreodoxa regia*. Fig. 1. Tree  $8\frac{1}{2}$  years old,  $\times \frac{1}{80}$  (approx.).

Fig. 2. The same tree,  $12\frac{1}{2}$  years old,  $\times \frac{1}{100}$  (approx.).

government officers, and the palm had passed into other ownership. The upper swelling had not appeared on March 13, 1926, but it was indicated on November 23, 1926, and clearly evident on February 22, 1928, when the

photograph, Fig. 2, was taken. Fig. 1 shows the palm in August 1923, about  $8\frac{1}{2}$  years old, and Fig. 2 in February 1928, about  $12\frac{3}{4}$  years old. The background is different in the second photograph, because the rubber trees on the other side of the hedge had been felled. Note the asymmetrical growth of the stem in Fig. 1, the swelling being greater on the more shaded side.

TABLE I  
*Growth of Oreodoxa regia*

| Leaf No. | Date of leaf-falls. | Interval (days). | Girth (cm.) |        | Percentage increase. |
|----------|---------------------|------------------|-------------|--------|----------------------|
|          |                     |                  | Initial.    | Final. |                      |
| 1.       | Nov. 8, 1919        | —                | 96.8        | 180.6  | 86                   |
| 2.       | March 7, 1920       | 120              | 97.5        | 217.5  | 123                  |
| 3.       | April 25, 1920      | 49               | 91.2        | 220    | 141                  |
| 4.       | July 18, 1920       | 82               | 91.8        | 207.5  | 126                  |
| 5.       | October 17, 1920    | 91               | 85          | 189.3  | 121                  |
| 6.       | March 13, 1921      | 147              | 81.2        | 174.7  | 115                  |
| 7.       | May 15, 1921        | 63               | 81.9        | 166.2  | 105                  |
| 8.       | July 13, 1921       | 59               | 77.2        | 161.5  | 109                  |
| 9.       | Nov. 9, 1921        | 119              | 82.5        | 157.1  | 92                   |
| 10.      | Feb. 24, 1922       | 107              | 82.5        | 154    | 87                   |
| 11.      | May 5, 1922         | 70               | 81.9        | 151.5  | 85                   |
| 12.      | July 22, 1922       | 78               | 83.8        | 150.3  | 81                   |
| 13.      | October 27, 1922    | 97               | 86.8        | 149    | 72                   |
| 14.      | January 28, 1923    | 93               | 86.8        | 147.2  | 69                   |
| 15.      | March 30, 1923      | 61               | 85.9        | 145.6  | 69                   |
| 16.      | June 6, 1923        | 68               | 88.1        | 145.6  | 65                   |
| 17.      | August 17, 1923     | 72               | 89          | 146.5  | 65                   |
| 18.      | Dec. 1, 1923        | 106              | 94          | 147.8  | 57                   |
| 19.      | Feb. 9, 1924        | 70               | 93.4        | 148.4  | 59                   |
| 20.      | March 24, 1924      | 44               | 94.3        | 149    | 58                   |
| 21.      | May 17, 1924        | 54               | 96.5        | 149.6  | 55                   |
| 22.      | August 12, 1924     | 77               | 99.3        | 150.9  | 54                   |
| 23.      | Nov. 13, 1924       | 72               | 102.2       | 152.2  | 49                   |
| 24.      | Dec. 22, 1924       | 70               | 102.8       | 152.2  | 48                   |
| 25.      | Feb. 22, 1925       | 62               | 101.5       | 150.9  | 48                   |
| 26.      | April 24, 1925      | 61               | 104.7       | 150.3  | 44                   |
| 27.      | —                   | —                | —           | 150    | —                    |
| 28.      | —                   | —                | —           | 149.6  | —                    |

Table I gives the date of the fall of each leaf, the number of days between successive leaf-falls, the initial girth at that leaf-scar or ring, the girth at the ring at the final measurement on February 22, 1928, and the percentage increase in girth over the initial girth (to the nearest whole number).

Four leaves fell in 1920, four in 1921, four in 1922, five in 1923, and six in 1924. The production and fall of leaves was thus more rapid as the tree grew taller, while the internodes were shorter, the lengths of successive internodes, beginning from the first ring being 15, 22.5, 25, 22.5, 21.5, 17.5, 16.25, 15.6, 14.7, 14.7, 14, 13.4, 13.4, 13.1 cm. These lengths remained unaltered. In general, the interval between the fall of successive leaves was greatest during October–March and least during April–September. At Peradeniya, January–March is the dry season, and the girth increment of deciduous trees is then at a minimum. The rains should begin in April, and April–October is the

season of most active growth, the rate falling in November–December. The fall of the leaf in *O. regia* no doubt depends to a great extent on the expansion of the stem, but it cannot be taken as a definite point for calculation, as the leaves do not all fall in the same condition. In general, the sheath separates from the stem all round at the same time, and the leaf falls while the sheath is more or less green and pliable, but in some cases the two sides of the sheath curl back gradually, so that the separation is prolonged, while sometimes the whole leaf may turn brown and dry up before it separates. It was not possible to correlate these differences with definite weather conditions. From April 24, 1925, to February 22, 1928, a further twenty leaves fell, the average interval being 52.2 days.

TABLE II

*Girth (cm.) of O. regia at each ring*

| Leaf-scar. | April 4,<br>1920. | April 3,<br>1921. | April 2,<br>1922. | April 1,<br>1923. | March 30,<br>1924. | March 29,<br>1925. | March 13,<br>1926. | Feb. 22,<br>1928. |
|------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|-------------------|
| 1.         | 114.3             | 150.6             | 168.7             | 180.6             | —                  | —                  | —                  | —                 |
| 2.         | 103.1             | 153.1             | 184.3             | 203.1             | 213.7              | —                  | —                  | —                 |
| 3.         | —                 | 135.6             | 173.7             | 200.8             | 214.7              | 218.4              | 219.3              | 220               |
| 4.         | —                 | 116.2             | 154.3             | 185               | 202.5              | 207.5              | 207.8              | 207.5             |
| 5.         | —                 | 96.8              | 130.9             | 161.8             | 181.8              | 189                | 189.3              | 189.3             |
| 6.         | —                 | 81.8              | 113.7             | 142.5             | 165                | 173.4              | 174.3              | 174.7             |
| 7.         | —                 | —                 | 103.4             | 130.9             | 152                | 163.7              | 165.6              | 166.2             |
| 8.         | —                 | —                 | 97.2              | 123.1             | 147.5              | 158.4              | 161.2              | 161.5             |
| 9.         | —                 | —                 | 91.5              | 117.2             | 142.2              | 154                | 157.5              | 158.1             |
| 10.        | —                 | —                 | 85.3              | 110.3             | 135.6              | 148.7              | 153.1              | 154               |
| 11.        | —                 | —                 | —                 | 104.7             | 130                | 145.3              | 150.6              | 151.5             |
| 12.        | —                 | —                 | —                 | 100               | 125.3              | 142.2              | 148.7              | 150.3             |
| 13.        | —                 | —                 | —                 | 95.6              | 120.3              | 138.7              | 147.2              | 149               |
| 14.        | —                 | —                 | —                 | 90.3              | 115.3              | 135                | 144.7              | 147.2             |
| 15.        | —                 | —                 | —                 | 85.9              | 109.7              | 130.3              | 142.2              | 145.6             |
| 16.        | —                 | —                 | —                 | —                 | 105.3              | 126.5              | 140.3              | 145.6             |
| 17.        | —                 | —                 | —                 | —                 | 102.8              | 124.7              | 139.7              | 146.5             |
| 18.        | —                 | —                 | —                 | —                 | 100                | 121.8              | 138.7              | 147.8             |
| 19.        | —                 | —                 | —                 | —                 | 97.2               | 118.7              | 136.5              | 148.4             |
| 20.        | —                 | —                 | —                 | —                 | 94.3               | 115.9              | 134                | 149               |
| 21.        | —                 | —                 | —                 | —                 | —                  | 114.3              | 131.8              | 149.6             |
| 22.        | —                 | —                 | —                 | —                 | —                  | 111.2              | 129.7              | 150.9             |
| 23.        | —                 | —                 | —                 | —                 | —                  | 109.7              | 127.8              | 152.2             |
| 24.        | —                 | —                 | —                 | —                 | —                  | 107.8              | 124.3              | 152.2             |
| 25.        | —                 | —                 | —                 | —                 | —                  | 104.7              | 122.2              | 150.9             |
| 26.        | —                 | —                 | —                 | —                 | —                  | —                  | 119                | 150.3             |
| 27.        | —                 | —                 | —                 | —                 | —                  | —                  | 117.2              | 150               |
| 28.        | —                 | —                 | —                 | —                 | —                  | —                  | 115.9              | 149.6             |

Table II gives the girth at each ring at intervals of one year in the first seven columns and nearly two years in the last. Increase in girth had practically ceased at the first ring on April 1, 1923, and at the second on September 28, 1924, roots beginning to appear in each case. At the third, increase in girth had almost ceased on March 13, 1926, the delay being probably due to initial root development, as at the fourth it had ended a year earlier. Measurements made on November 28, 1926 (not given in the Table), and February 22, 1928,

show that no increase in girth occurred at rings 4 to 13 during that period, 1 year 3 months. Girth increment had therefore ceased by the former date up to the thirteenth ring. It is evident that *the increase in girth at any given ring in O. regia is a transient phenomenon and the region of active growth in thickness gradually recedes from the base of the stem.*

The growth curves for the several rings are similar, all the curves, in general, being in the same direction at any given time. Growth at each ring was rapid when the leaf fell, but gradually decreased and after about four years ceased. There was a maximum in April 1920 and a fall to a minimum in March 1921. 1921 was a drought year, the rains being well below the average until August; as regards the eight rings then visible, there was a maximum growth in April, but it fell to a minimum in July, after which, following heavy rains in August and October, it rose to maxima in September and November, thence falling to a minimum in January 1922. After that, growth rose to a maximum in June 1922 and fell to a minimum in January 1923. In the latter year there was a partial failure of the early rains until June; the growth curves show a maximum in April 1923 and another in September–October, with a minimum in January 1924. A maximum is shown in April 1924 and a minimum in January 1925. Thus, in general, growth followed the rule which obtains for dicotyledonous trees at Peradeniya, but *O. regia*, at least in its younger stages, appears to be more susceptible to weather conditions, and drought in the first half of the year may cause a fall in the growth rate in July, with a subsequent rise later in the year after the rains have come.

The initial measurement at each ring shows that the girth of the bud decreased regularly upwards at first. In the Table the measurement given is that at the leaf-scar after the fall of the leaf, but those made previously at the base of the leaf-sheath show the same effect. After the eighth leaf-fall, however, the girth of the bud began to increase, and this increase continued with slight variation during the remainder of the measurements. Thus the initial girths in column 3 of Table I increase from 82.5 cm. at ring 9 to 104.7 cm. at ring 26. The percentage secondary increase at each ring is given in column 4, which shows a decrease upwards after the second ring, though it must be borne in mind that growth at the upper rings was no doubt not complete. However, although the secondary increase at the twenty-fourth ring on February 22, 1928, was only 48 per cent., as against 71.6 per cent. at the thirteenth ring, the girth at the twenty-fourth ring on that date, 152.2 cm., exceeded the girth at the thirteenth ring, 149 cm. (growth finished). It would seem a valid conclusion, therefore, that *the formation of the barrel is due to the combination of two factors, the greater initial diameter of the bud and the decrease in secondary growth.*

On November 28, 1926, the thickest part of the barrel was at rings 18 and 19; on February 22, 1928, it was at rings 23 and 24.

Notwithstanding the enormous increase in girth, there is no more than a very minute superficial splitting of the outer tissues of the stem.

On several occasions measurements were made on the same day in the

early morning, at midday, and in the evening to ascertain whether there was any diurnal variation in girth. No such effect was observed.

It is regretted that it was not possible to carry these observations further. The results obtained may serve to indicate when and where examinations should be made of the internal structure of the tree.

My thanks are due to Dr. G. Bryce for continuing the measurements when I was absent on leave in 1920-1.

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# Studies in Growth Analysis of the Cotton Plant under Irrigation in the Sudan

## III. A Comparison of Plant Development in the Sudan Gezira and in Egypt

BY

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With eight Figures in the Text

### INTRODUCTION

COMMENT has frequently been made on the difference in the appearance of the cotton crop in Egypt and the Sudan Gezira. Thus Bailey (1925) wrote: 'To a new-comer who has had an opportunity of studying the Egyptian cotton plant in Egypt, the appearance of the plant as seen growing in the Gezira is disappointing.' Elsewhere, writing of the comparative 'legginess' of the Sudan crop, he remarked: 'The most striking difference lies in the suppression of fruiting branches on the lower portion of the main stem.'

It is natural that observers should measure the efficiency of the Sudan crop against that of Egypt, for the two crops, although about twelve hundred miles apart, are irrigated from the same river and comprise a single type of cotton, i.e. Egyptian (*Gossypium barbadense*), and perennial irrigation of the crop was more than a century old in Egypt when in 1925-6 the Sennar Dam was built to irrigate the Sudan Gezira.

Balls (1912), ending his book, 'The Cotton Plant in Egypt', said: 'There is a long field of operations in which our present results may be re-examined, stretching from the Mediterranean into the heart of the Sudan.' Again (1917), discussing the limitation of conclusions based on results from a single centre, he wrote: 'The ideal method would have been to obtain data from a number of permanent observation stations, so scattered over the country as to provide a fair sample of the whole, and preferably subjected to the ordinary cultivation and irrigation régime of the site and season.'

The work which Balls started so successfully in Egypt was extended to the Sudan by Lambert when in 1922 he began recording developmental observations in the Gezira. On his retirement these records were continued by the author, and the data from these records and from several multiple-factor experiments made by the author in both the Sudan and Egypt form the basis of the present comparison and seem to fulfil very well the conditions set forth above as desirable by Balls. Details of the experiments have already been published: for the Sudan Gezira by Lambert (1928 to 1937) and Crowther

(1934, 1941), and for Egypt in bulletins of the Royal Agricultural Society of Egypt (Crowther, 1936, 1937a).

It is natural to expect that Egyptian cotton will grow most successfully in Egypt, and to use the physiological behaviour of the plant there as a standard against which to measure its growth in other climates. The comparison between the Sudan and Egypt was easy during the years 1918–35, since Sakel, selected in Egypt, was the principal long staple variety in both countries, seed for the commercial area of the Gezira often being imported from Egypt. Recently in both countries new strains have been selected of which the geographical range is restricted, and in future physiological comparison may be impaired through lack of a variety common to both countries.

#### DESCRIPTION OF THE DATA

The Sudan data comprise fifteen years' results from one permanent observation plot at the Gezira Research Farm, established by Lambert in 1927–8, the results from which have already been published (1928–37). To gauge how far the results from this 'Old' plot were applicable to Gezira conditions generally, three 'New' permanent experiments were started in 1938–9, namely, at Turabi, 57 miles north, at Hag Abdulla, 29 miles south of the Old observation plot, and a home plot adjacent to it. These are well distributed over the Gezira scheme, and when started were on land representative of their immediate surroundings. The agricultural practice employed on all these plots has been maintained as near as possible to that of the commercial crop, and, despite inevitable differences, the seasonal fluctuations of growth and yield are highly correlated with those of the neighbouring crop (Crowther, 1941). While methods of sowing, weeding, irrigation, and picking are almost identical with those of the commercial area, there are differences in rotation, variety, and manurial practice. The Old observation plot is on a 3-year rotation, namely cotton–*Dolichos lablab*–fallow,<sup>1</sup> while the commercial area, originally on a 3-year rotation, has been converted to a 4-year one with 2 years' fallow before cotton. The New plots are on a 3-year rotation, but the *Dolichos* has been replaced by fallow, giving 2 years' fallow before cotton, as in the commercial crop. Sakel (*G. barbadense*) was grown throughout the Gezira in 1927–8 and consequently a particular strain (Massey's selected Domains Sakel) has been adopted as standard for all observation plots. The New plots include also a second variety the same as that of the surrounding crop, which provides a link between that crop and the experiments. The Old observation plot is manured with ammonium sulphate (300 rotls per feddan,<sup>2</sup> 6 weeks after sowing) to reduce the effects of soil factors and thus facilitate the measurement of climatic effects, but the bulk of the commercial crop is not manured. This manuring, with its cumulative residual effects, together

<sup>1</sup> The fallow land is left uncultivated, except that weeds are hand-hoed to check their seeding.

<sup>2</sup> 1 rotl = 0.99 lb. = 450 gm.; 1 kantar = 315 rotls : 1 feddan = 1.038 acres; 300 rotls per feddan = 286.3 lb. per acre.

with the presence of Dolichos in the rotation, have occasioned a progressive increase in yield not reflected in adjacent plots. Hence most of the growth data for the Sudan presented in this paper have been collected from a crop which, over a period of 15 years, has yielded above the Gezira average for the Sakel variety. The extent of this manuring effect has been assessed from the New experiments, for they provide data from unmanured as well as from manured plots. The writer, from his examination of the New observation plots, is satisfied that conclusions based on the data of the Old observation plot are valid for large areas of the commercial crop.

For Egypt the principal data are derived from a series of 12 multiple-factor field experiments, covering the 3 seasons of 1934-6 (Crowther, 1936; 1937a). These were well distributed over the Nile Delta and included also one experiment south of Cairo. Being situated mostly on private estates they may be taken as representative of the commercial crop. Cotton was usually in a 2-year rotation comprising also wheat, either maize or rice, *Trifolium alexandrinum*, and rarely more than 3 to 4 successive months' fallow. The commercial varieties then most widely grown were included, and the Egypt data, therefore, are averaged from several varieties including Sakel, whereas the Sudan data are based on Sakel only. In the present comparison variety is thought to have exerted a marked influence only in the case of boll characters, and in the section on that subject data from Sakel plants only are employed for both countries.

The comparison is facilitated by the uniformity of technique of field procedure and crop measurement as carried out by one and the same experimenter in both countries. The observational methods employed include those developed by Balls (1915a) and Gregory (1921, 1926). When the crops are described as 'Sudan' or 'Gezira' and 'Egypt', they refer respectively to those of the Sudan Gezira and the Egyptian Delta.

#### SOIL AND WEATHER IN THE TWO COUNTRIES

The Gezira Research Farm (lat. 14° 24' N., long. 33° 30' E.) is situated at Wad Medani, 1,150 miles south of the mid-point of the delta (Qorashia, lat. 30° 15' N., long. 31° 7' E.), and is roughly half-way between Qorashia and the Equator. The irrigation water for the Gezira scheme is provided by the Blue Nile. This joins the White Nile at Khartoum, 110 miles downstream from Wad Medani, and the two rivers form the Nile which irrigates Egypt.

The soil of the Gezira has been described by Greene and Snow (1939) as 'heavy clay, strongly alkaline, of low permeability to water and low in content of nitrogen and humus'. There is marked swelling and cracking of the soil and very slow water movement. In Egypt most of the cotton soil is fairly heavy clay, but unlike that of the Gezira has a medium-to-high permeability, allowing of free water movement. This contrast in permeability is well illustrated by the differences in the water-table. In the Gezira there is no free water within reach of cotton roots, and after irrigation the soil is wettest at the surface and becomes progressively drier in the deeper layers of the root

zone. In Egypt the water-table, often only 1 to 2 metres below the surface, frequently fluctuates with changes in the level of the Nile, even at a distance from the river.

Before consideration of the weather in the two countries, reference must be made to the time of year when the cotton crop is grown, for this differs markedly, the Sudan crop being a winter one and the Egypt a summer one. Table I gives the dates of important operations for the Sudan Gezira Old observation plot and for the average of the Egypt experiments. For the commercial crop sowing starts in the Gezira from about August 10 and in the delta in late February, so the dates in the experiments may be slightly later than the average in both countries.

TABLE I  
*Details of Agricultural Operations*

| Operation.                                 | Date   |         | Age of crop (days)  |        |
|--|--|---------|---|--------|
|  | Sudan.   | Egypt.  | Sudan.  | Egypt. |
| Sowing . . . . .                           | Aug. 19  | Mar. 8  | 0   | 0      |
| Thinning . . . . .                         | Oct. 2   | Apr. 21 | 44  | 44     |
| Picking, first . . . . .                   | Jan. 1   | Aug. 19 | 135   | 164    |
| „ last . . . . .                           | Apr. 15  | Oct. 7  | 239   | 243    |
|  |  |         |   |        |
|  | Sudan  |         | Egypt   |        |
| Irrigations (after thinning) . . . . .     | 14, at 14-day intervals  |         | 9 or 10, at 11- to 16-day intervals                                       |        |
| Crop spacing (2 plants per hole) . . . . . | Between ridges 80 cm.<br>Between holes, 50 cm.<br>(0.40 sq. m. per hole) |         | Between ridges, 59 cm.<br>Between holes, 25 cm.<br>(0.15 sq. m. per hole) |        |

Harvesting begins in the Gezira when the plants are younger than in Egypt, but the picking season in the Gezira is protracted; in both countries the plants are uprooted 7-8 months after sowing. Partly because of the long picking season and partly because the seed-cotton is easily shed from the ripe bolls, the Sudan practice is to make regular fortnightly pickings totalling at least 7 in the season, in contrast with the usual 2 pickings made in Egypt.

Irrigations are given regularly in both countries, but the interval between them tends to be shorter in the Sudan. In the spacing of the crop there is a marked difference, for whereas in the Gezira there is an average of 5 plants per sq. m. after thinning, in Egypt the average is 13.3 plants or more than 2½ times as many per unit area. This contrast was not so great formerly, for Balls (1915a) gave the normal spacing along the ridge as 45 cm. and Templeton (1932) gave the optimum as 35 cm. Counts made by the writer on random samples of the commercial crop in Egypt in 1937 gave spacings ranging from 26 to 29 cm. between holes along the ridge.

The data for the weather are presented in Fig. 1, which shows the normals for the Gezira Research Farm, Sudan, and for Qorashia, Egypt. They have been extracted from 'Climatological Normals' (1938), prepared by the Physical Department, Cairo, except those for wind and hours of daylight, which are

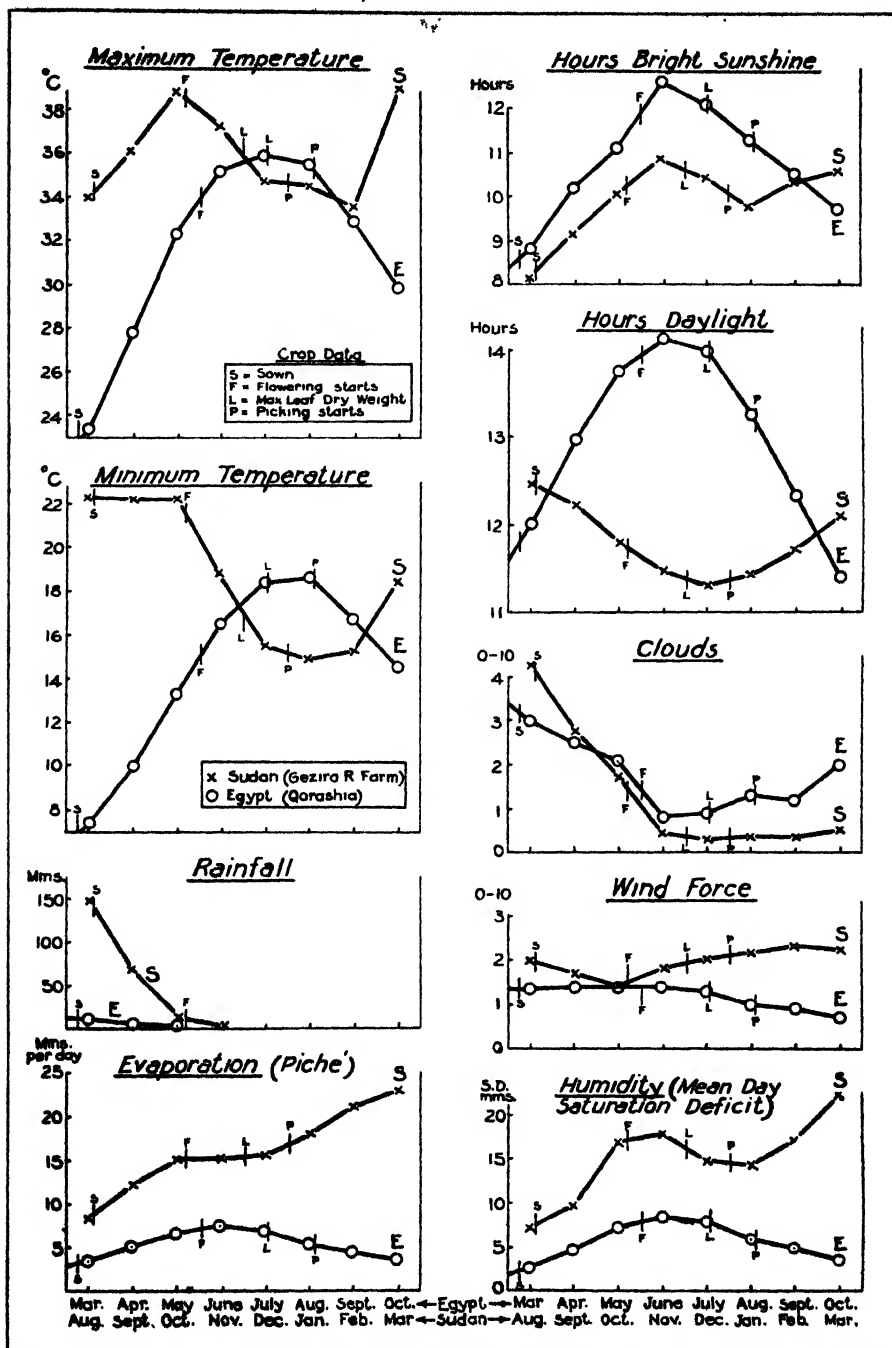


FIG. 1. Graphs of meteorological data for Gezira Research Farm, Sudan, and Qorashia, Egypt, grouped to show climatic conditions at comparable stages of crop growth.

taken from the summary by Williams (1924) of the climates of cotton-growing areas. The values for wind and clouds, though based on observers' estimates on a 0-10 scale, are sufficiently reliable to illustrate the major differences between the two stations. The data are plotted according to the method used by Williams, i.e. the March values for Egypt and the August values for the Sudan Gezira are superimposed along the abscissae. This arrangement brings into relief the sharp contrast in weather experienced by the plants of the two countries at successive stages of growth. The times of sowing, flowering, maximum leaf dry-weight, and picking are indicated on all graphs. It is realized that the actual climate prevailing for the two crops differs from that indicated in Fig. 1 which is based on readings from standard screens, but, for the purposes of this comparison, it may be assumed that the difference in the climates of the two crops is of a magnitude similar to that of the data from the screens.

The Sudan crop, though a winter one, experiences temperatures, averaged over the whole period during which the plants are in the ground, higher than that for the Egypt crop. The differences are especially marked at sowing time; for while minimum temperatures in the Sudan average 22° C., in Egypt they average about 8° C. Night temperatures remain high in the Sudan until the beginning of flowering; subsequently they diminish, the curves for minimum, as well as those for maximum, temperatures in the two countries converging. By the time of the greatest leaf dry-weight, both maximum and minimum temperatures are appreciably *higher* in Egypt than in the Sudan, and they continue so until picking begins, after which the curves diverge rapidly at the approach of summer in the Sudan and winter in Egypt.

The Gezira receives rain from the summer monsoon, which is especially heavy in July and August, the annual total at Wad Medani being normally 400 mm. The delta receives only the light rains of the Mediterranean winter, and the normal at Qorashia is 61 mm., nearly all falling between December and March. Thus in both countries the cotton is sown towards the end of the rainy period. This means that sowing takes place during the most cloudy weather and that leaf dry-weight reaches its maximum during the least cloudy period. Winds are consistently stronger in the Sudan and these, combined with lower humidity, lead to a persistently higher rate of evaporation. The difference between the countries is least during the rains, i.e. at the time of sowing, and increases progressively during the season. Thus in Egypt the cotton bolls open during weather of light wind, low evaporation, and falling temperatures, whereas in the Sudan harvesting takes place at a time of considerable wind and high evaporation and on a rising temperature curve.

Variation in the length of day is in the Gezira only about 1½ hours during the year, whereas in Egypt it is nearly 4 hours. Arising from the difference in date of sowing, the period of maximum dry-weight coincides with the *shortest* days in the Sudan and the *longest* days in Egypt. On this subject Williams (1924) comments: 'Cotton in the Sudan is probably grown with less

daylight than any other country, as it is a winter crop further away from the Equator than any other winter grown cotton.'

Damage by pests and diseases in the experimental data did not greatly interfere with the comparison of growth in the two countries. In the Gezira, blackarm (*B. malvacearum*) in some seasons caused premature defoliation and, where severe, loss of developing fruits. Among the standard practices which Lambert adopted for the Old observation plot at its inception was a somewhat late sowing date, chosen with the express purpose of reducing interference by blackarm, which he had observed to be much less on late sowings. Leaf curl, to which the Sakel variety is susceptible, prevented the normal development of the later-formed leaves and fruits. The effects of blackarm and leaf curl on the present comparison can be ignored unless specific reference is made, since either the data used for the Gezira were taken from seasons when such damage was negligible or else the effect was minimized by the use of the average of up to fifteen seasons, in the majority of which most of the plants observed were healthy. In Egypt, leaf worm (*Prodenia litura*) was present in great numbers on most of the experiments and was controlled by frequent hand-picking of egg-masses. Pink bollworm (*Platyedra gossypiella*) was abundant late in the season in all years, but the pest need only be considered in the data of boll development.

#### FIELD GERMINATION

In the Gezira germination was much quicker than in Egypt, as may be seen from Table II, which gives comparable data from various sources for the number of days between sowing and the appearance above the ground of at least half the full stand. The date of sowing was taken in all cases as the day when the seed was first wetted, in the Sudan when the seed was placed in the ground and in Egypt when the dry-sown seed was first irrigated. All are for sowings made at the normal date.

TABLE II

#### *Number of Days for Field Germination*

| Sudan.                |   |   |   | Egypt.                      |   |   |       |
|-----------------------|---|---|---|-----------------------------|---|---|-------|
| Old Observation plot  | . | . | 4 | Balls (1912)                | . | . | 12    |
| New Observation plots | . | . | 4 | Templeton (1924)            | . | . | 12    |
|                       |   |   |   | Multiple-factor experiments | . |   | 11-20 |

The Gezira crop appeared after 4 days, the Egypt one after about 12 days. Balls as early as 1912 stressed the control of germination in Egypt by low temperatures at the normal time of sowing, i.e. February and March. Where his experimental sowings were made in mid-April, when the temperature of the seed-bed was 23° C., the cotyledons took 5 days to appear above ground, in contrast with 11 days for mid-February when the temperature was 14° C. Camp and Walker (1927) found that the rate of germination of cotton for temperatures varying between 15° C. and 35° C. agreed very well with



van't Hoff's rule. In the present experiments, assuming soil temperatures in both countries to be related similarly to minimum air temperatures, a difference of  $14.8^{\circ}\text{C}$ . would indicate, for a temperature coefficient of 2.0, a period of 11.8 days for field germination for Egypt as compared with 4 days for the Sudan, an estimate which is close to that found by observation.

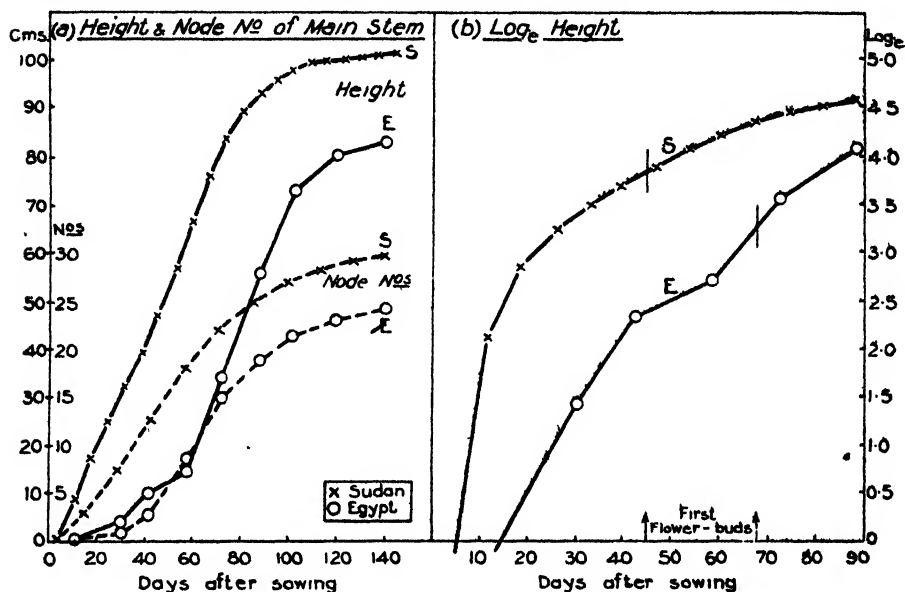


FIG. 2. Graphs showing (a) heights and nodes and (b) natural logarithm of height, of main stem. Sudan curves averaged from 15 years data for heights, and 11 years for nodes; Egypt curves based on 1 experiment. (In this and following figures the arrows indicate the time of the appearance of the first flower-buds.)

The Egyptian peasant, to lessen the delay in germination from low temperatures, always makes his cotton ridges run east to west and sows the seed half-way up the south face of the ridge where the soil is sheltered from the north wind and there is greater radiation per unit area.

#### DEVELOPMENT OF THE MAIN STEM

Fig. 2 (a) shows the height of the main stem and the total number of nodes, recorded at regular intervals after sowing. The Sudan data from the Old Observation plot are derived from the averages of 15 seasons for heights and 11 for nodes; those from Egypt are from a single experiment at Bahtim made in 1934, the average of 3 varieties. The latter data are not necessarily representative of the final height attained in Egypt, but the general shape of the curve, reflecting retarded early growth followed by a period of rapid development, is typical.

In the Sudan, height and number of nodes increased rapidly from the earliest stage. By contrast in Egypt, as with field germination, the early

development of the main stem, measured both by height and by node numbers, was greatly retarded; for example, at 40 days the plants in the Sudan were 4 times as tall as those in Egypt and possessed 4 times as many nodes.

The final height attained in Egypt, averaged from 10 experiments, was 72.1 cm. and the final node number 23.1. By comparison, in the Sudan the Old observation plot averaged 103.1 cm. for final height and 29.5 for node number, while unmanured cotton on the New observation plots averaged, over a different set of seasons, 101.2 cm. and 29.4 respectively. The height and node number of the Sudan Gezira crop were therefore considerably greater than those of the Egyptian crop. This difference arose primarily from the closer spacing in Egypt, and secondarily from the shorter habit of growth of new Egyptian varieties as compared with Sakel.

Increase in height is a complex function. Balls (1915) stated: 'There would seem to be a real distinction between the development of nodes and the extension of internodes.' Crowther (1934) found that under the weather conditions of the Sudan Gezira increase in node number, being the result of meristematic activity, was a function of nitrogen supply, whereas extension growth, as expressed in internode length, was primarily a function of water supply. Increase in height is the product of the two.

The difference between the two countries in the rate of production of nodes in the early stages can be explained in terms of temperature. Thus in the Sudan during the 40 days after germination, when the mean minimum temperature was 22° C., 13 nodes were produced. In Egypt for the same period, when the mean minimum temperature was 10° C., only 6 were produced. This gives a temperature coefficient of 1.8 for an increase of 10° C., as compared with the value of 2.0, which would be expected from van 't Hoff's rule. Balls (1912) stressed the limitation of height increase by low night temperatures in Egypt during early growth, and this effect of temperature upon the rate of node production is itself sufficient to explain much of the difference between the Sudan and Egypt in the rate of height increase.

The natural logarithms of the height data for the Sudan and Egypt are plotted in Fig. 2 (*b*). From germination to flowering, i.e. until 3 weeks after the date of the appearance of the flower-buds (as indicated in the figure) increase in height in Egypt was approximately logarithmic, but, in view of the progressive rise in temperature from a low initial value, it is impossible to decide to what degree the increasing temperatures contributed to the maintenance of exponential growth. In the Sudan there is no definite indication in Fig. 2 (*b*) of exponential growth over any period. The relative rate of height increase was not constant, as it must be with exponential growth, but fell progressively with time. This fall was such that the time curve for heights tended to be linear instead of exponential from germination to the opening of the flowers, 2 months later. Heath (1932) found that the same time curve for rain-grown cotton in South Africa was approximately exponential, and Afzal and Iyer (1934) found an exponential curve for irrigated cotton

in the Punjab. Thus the data for Egypt are in agreement with those from other countries, whereas the Sudan data are exceptional.

Balls (1915) suggested that a temperature of 32° C. was near the optimum for the growth of cotton. In the Sudan at sowing the maximum day temperatures are near this optimum, but subsequently they increase to a mean monthly maximum of 38° C. 2 months later, so that the plants experience progressively longer periods at supra-optimal temperatures. In view of Gregory's findings with *Cucumis* (1928), that at supra-optimal temperatures a 'time factor' operates tending to reduce relative leaf growth rate, it might be postulated that the height curve in the Sudan reflected the limiting effect of high temperature. Moreover Balls in Egypt stressed the depressing effect of high temperatures upon rate of elongation of the main stem, and Crowther (1933-4) found from daily observations in the Sudan a significant negative correlation between increases in height and in maximum temperatures during September and October. These temperatures, however, appear not to be critical in determining the final height attained, for no correlation has been detected between seasonal differences in final height and corresponding differences in temperature.

There is, on the other hand, when a number of seasons are compared (Lambert, 1936-7; Crowther, 1941), a highly significant correlation between height measured at 6 weeks after sowing and final yield, and both are correlated with nitrogen percentage in leaf dry matter as early as 7 days after sowing. In Egypt also, within individual experiments, nitrogenous manuring increased final height as well as yield. Thus, though rate of height-increase is affected by temperature, the final height attained by the plants is governed to a considerable degree by an internal factor associated with nitrogen supply.

#### FRUITING BRANCHES AND POTENTIAL CROP

The flowers of the cotton plant are borne on sympodial branches arising from the axils of the leaves on either the main stem or on lateral monopodial branches. The latter are not considered in the present paper, since under the normal spacing of both countries only a small part of the crop is produced on the monopodia.

##### *Distribution and development of fruiting branches.*

Fig. 3 (a) shows the distribution of the fruiting branches up the main stem, the values being the totals for zones of 5 nodes. The data available for the Sudan are for the 4 years of the Old observation plot immediately following the writer's return from Egypt, namely 1938-9 to 1941-2 inclusive. The variety grown was Sakel, and as mentioned above the crop yields were above average. The Egypt data are averaged from 10 experiments, made in the two seasons 1935 and 1936. They comprise the average of 5 varieties and are representative of the delta crop. Aboul Ela (1932) stated that Sakel carries fruiting branches starting at about the same node as Giza 7, but at a slightly

higher node than Maarad. Thus the inclusion of Maarad among the varieties in the Egypt data led to the initiation of fruiting, for the average of the varieties, at a fraction of a node lower than for Sakel alone. The records are for a comparable stage in both countries, shortly before the opening of the bolls, i.e. early December in the Sudan and late July and early August in Egypt.

In both countries the development of fruiting branches was similar in the two lower zones; but higher up the stem their production, in Egypt, was drastically curtailed. In Egypt, too, the number of flower-buds produced on a single branch was much lower in all the important zones. This is shown in Fig. 3 (b), which gives the total number of nodes on the fruiting branches, each node carrying initially one flower-bud. These totals necessarily take no account of any nodes of the terminal portions of fruiting branches which were shed prior to the observation period. Such shedding is not known to be appreciable in either country, but shedding of flower-buds and bolls on the less distal parts is appreciable in both countries and is included in these counts, see Fig. 3 (c).

The final result of these differences in the production and extension of branches, with the shedding of flower-buds and bolls, is the distribution up the main stem of the potential crop (buds, flowers, and bolls); see Fig. 3 (d) where the production is given separately for each node of the main stem. The first of these nodes to carry mature fruit was slightly lower by, at the most, 2 nodes in Egypt than in the Sudan, but, in general, the plant's crop up to the 10th node was distributed similarly. In the Sudan the upper part of the plant developed much more than in Egypt, and, whereas in Egypt the node which carried the greatest crop was the 14th, in the Sudan it was the 17th.

If the numbers of plants per unit area were the same in both countries, these curves would show the relative distribution of the potential crop on the plants. But, as shown in Table I, there were  $2\frac{1}{2}$  times as many plants per unit area in Egypt as in the Sudan Gezira, and this closer spacing profoundly alters the comparison. Fig. 3 (e), giving the total fruits carried at each node of the main stem *per square metre of field*, shows a similar total number of fruits comprising the entire crop in both countries, but a marked concentration of fruits on the lower nodes in Egypt. The importance of these lower nodes is illustrated in Table III, which gives the percentage of the crop borne in the different zones of the main stem.

TABLE III

*Percentage Distribution of the Potential Crop up the Main Stem  
(per unit area of field)*

|   | Sudan. | Egypt. |
|---|--------|--------|
| (a) At or below the 10th node . . . . . | 4.5    | 12.5   |
| (b) On the 11th to 20th nodes . . . . . | 57.4   | 72.0   |
| (c) Above the 20th node . . . . .       | 38.1   | 15.5   |

The lower nodes in Egypt carried nearly 3 times the amount of crop borne

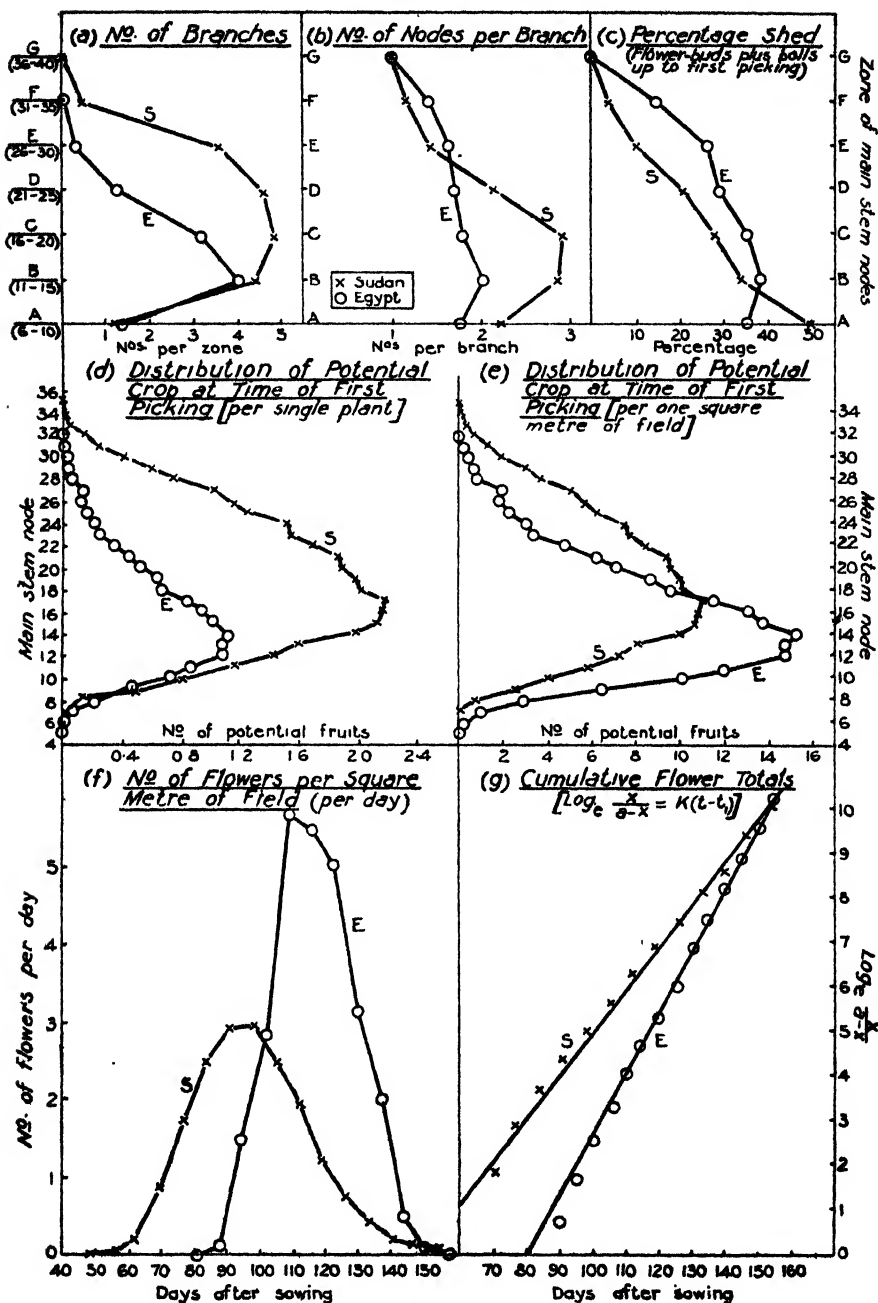


FIG. 3. Graphs showing (a) number of fruiting branches per zone of 5 nodes of the main stem, (b) number of nodes per fruiting branch, each node bearing initially a flower-bud, (c) percentage of flower-buds shed up to time of first picking, (d) distribution per single plant of potential crop (buds, flowers, and bolls) at first picking, (e) similar to (d), but distri-

by the corresponding nodes in the Gezira. More plants per unit area resulted in more plants fruiting simultaneously on the lower nodes. Not only was the bulk of the Egypt crop borne on lower nodes, but also it was concentrated on fewer nodes. This led to a flush of open bolls at harvest and explains why in Egypt there are heavy pickings for a short period, whereas in the Sudan the crop arrives more slowly and the picking season is prolonged.

The contrast between the Sudan and Egypt in the development of fruiting branches has received considerable attention. For instance, Bailey (1925) stated: 'In Egypt the first fruiting branch appears on the average in the axil of the seventh true leaf. In the Sudan plants of the same variety, grown in fact from the same seed, rarely put out fruiting branches before the twelfth to fourteenth leaf has been reached.' It was presumably that statement by Bailey which led Mason (1928), when considering the 'unsolved problems awaiting the physiologist', to say: 'Sakel cotton, for instance, in the Sudan produces its fruiting branches much higher up the stem than in Egypt, thus causing a considerable delay in the setting of the crop. . . . The causes of these and similar alterations in habit are quite unknown.' Leake (1928), discussing the importance of air humidity, treated the problem of fruiting branches in the Sudan at some length and concluded: 'The conditions under which the plant is growing at the time when the first fruiting branches are forming appear to be critical; a change, so small as to be far from obvious, being sufficient to produce a marked change of habit.' This conclusion was based on observations by Bailey (1928).

Bailey's comments were of a preliminary nature, and at the time (1925) he stated of the Gezira crop: 'I should like to emphasize now our need for more detailed observations on the actual development of the plant. Our ignorance on this matter is very considerable indeed', and it was with Bailey's support that Lambert was able to initiate the observation plot in 1927-8. As a result of 15 years' data from this plot it is concluded that the plants examined by Bailey lacked the lower fruiting branches primarily because of blackarm damage. The reports of the Gezira Research Farm for 1924-5 and 1925-6 expressly stated that blackarm was severe, whereas in 1926-7 blackarm was reported as 'practically non-existent'. Regarding the latter season, Bailey (1928), commenting upon the contrast in the appearance of the crop compared with previous years, stated: 'The difference lay in the fact that the phenomenon of suppression of the lower fruiting branches was practically absent.' Finally, in the following year, 1927-8, Bailey (1929) himself concluded that blackarm was responsible for considerable 'denudation' of fruiting branches in that season. Thus it seems likely that early commentators on the morphological habit were misled by their failure to recognize the extent to which

bution per square metre of field, (f) number of flowers produced per day per square metre of field, and (g) cumulative flower totals expressed on logarithmic basis.

Sudan curves in (a) to (e) averaged from 4 years', and in (f) and (g) from 12 years' data; Egypt curves in (a) to (c) averaged from 10 experiments made during 2 years, in (f) from a single experiment (Bahtim, 1934), and in (g) from an experiment conducted by Prescott (1921).

blackarm damage interfered with the normal expression of plant habit. Lambert, on the contrary, devoted most of his first report of 1922-3 to his observations on the effects of blackarm on the crop, and on them based his selection of a rather late sowing date for the observation plot.

The data presented in this comparison of the two countries show that there was in the Sudan a delay in the formation of fruiting branches amounting, at the most, to 2 nodes, and this delay, it is suggested below, can be ascribed directly to temperature. The subsequent production of fruiting branches was governed primarily by crop spacing, competition between plants being more severe in Egypt than in the Sudan.

Regarding the influence of temperature upon the formation of fruiting branches Balls (1912) suspected the existence of the inhibiting effect of high temperature; Zaitsev (1927) concluded, 'Height of the first sympodium is influenced by certain external factors amongst which is temperature'; and Bailey (1929) showed from experiments on plants of various sowing dates growing in boxes that high temperatures suppressed the formation of fruiting branches. Though no specific experiments have been made in the Gezira to study this effect, evidence is available from field observations on the Old observation plot. Counts of the lowest node on the main stem to bear a fruiting branch whereon the flower-bud survived to maturity (lowest sympodial node) were made for 10 years, and the average node number approximated closely to 10.0, when the cotyledonary node was counted as zero. Although this value was representative of the crop, individual plants fruited at lower nodes, as seen in Fig. 3 (*d*). Even despite the small range of temperature involved when comparing the means for the same month in successive years, the correlation of the position of the lowest fruiting branch (i.e. sympodial) node with mean September maximum temperatures is  $+0.668$  (significance  $P < 0.01$ ). The regression equation for lowest sympodial node in terms of temperature is

$$y = 0.3953x - 4.03,$$

where  $y$  is the number of nodes and  $x$  the mean September maximum temperature ( $^{\circ}\text{C}.$ ), September covering the period of 2 to 6 weeks after sowing during which the initials of fruiting branches are laid down. Applying this equation to the Egypt data for the corresponding period of 4 weeks before the appearance of flower-buds, when the mean monthly maximum temperatures for the particular seasons in which the experiments were conducted was  $31.6^{\circ}\text{C}.$  in Egypt as compared with  $35.5^{\circ}\text{C}.$  in the Sudan, the position of the lowest sympodial node is evaluated as 8.5 in Egypt, as compared with 10.0 in the Sudan—a difference of 1.5 nodes. This estimate is comparable with the 2 nodes already noted, the latter being slightly inflated by varietal differences.

#### *Shedding of flower-buds and bolls.*

The causes underlying the shedding of flower-buds and bolls in the cotton crop have been discussed at length in many countries, and Egypt is no excep-

tion. Balls (1912) concluded that shedding of both buds and flowers in his experiments arose from root asphyxiation which destroyed the water equilibrium between root and shoot. He also stressed that shedding varied especially with change in water supply, being heavier with water shortage and lighter after an irrigation. Whereas Balls (1915*a*) maintained that more flowers were shed than flower-buds or bolls, Prescott (1924*a*) and Bailey and Trought (1927) concluded that most of the shedding occurred *before* flowering, usually whilst the buds were small. The latter suggested that the external causes which brought about this bud-shedding had yet to be determined with certainty.

In the Sudan Bailey (1930) found that shedding, as in Egypt, occurred mainly as flower-buds but the average age of the buds was slightly higher than in Egypt. He concluded that the period of heaviest shedding was mid-October to mid-November, which coincided with a period of especially low humidity. Earlier (1929) he had failed to detect any increase in shedding just before or just after an irrigation, but found some indication of additional shedding from high temperature.

As for the data presented in this paper, most of the shedding recorded in Fig. 3 (*c*), that is, up to the opening of the bolls, was of flower-buds. In Egypt there was subsequently considerable shedding and death of bolls at the upper nodes through premature cessation of irrigation, to hasten opening of bolls, and from bollworm attack. Proportionally, shedding of flower-buds in both countries was greatest at the lowest nodes and decreased progressively up the plant. Considering all nodes, this shedding was less in the Sudan than in Egypt.

This last result is the reverse of expectation on a basis of water strain. Not only is the soil of the Sudan Gezira less permeable to water than that of Egypt, but also the saturation deficit in the atmosphere of the Sudan is greater at all times than in Egypt, and especially at flowering. Balls suggested that shedding is likely to occur when temperature exceeds 37° C., but the mean weekly temperature at the Gezira Research Farm normally exceeds 37° C. for 5 successive weeks during the main growth period, including the stage of the maximum production of flower-buds. Balls (1912) stated: 'In the Sudan, however, spells of hot dry wind are generally recognized as being precursors of shedding epidemics', but this probably referred to areas other than the Gezira which was only in its infancy then, for in the Gezira winds are light at the time of the formation of flower-buds.

The present study of the two countries has produced no reason for modifying the view on shedding in the Sudan put forward by Gregory, Crowther, and Lambert (1930), namely that the number of flowers laid down depends on nitrogen relations, and that the continued growth of the bolls depends on carbohydrate supply. Just as a cereal such as barley produces more tillers than it can carry through to heads, so the cotton plant produces more flower-buds than it can bring to mature bolls; for, as will be seen from Fig. 6 (*d*), the plant in Egypt had, during the period of flower-bud formation, a higher



concentration of nitrogen than its Sudan counterpart, and flower-buds were laid down more rapidly. Subsequently, because of the larger number of developing fruits in Egypt per unit area of field, competition for assimilated products was the more intense and shedding, therefore, the greater.

Thus, in the Sudan, whether or not on particular days 'bud-shedding was increased by high temperatures or by water strain, there was no evidence that the total amount of shedding was increased by these causes nor were final yields affected. Such bud-shedding, and the additional shedding which might occur from light incidence of pests or diseases, was, it is suggested, partially or wholly offset by reduced shedding from nutritional causes. Where shedding was both early and abnormally severe, the final yields may have been actually *augmented* by the postponement of the check which fruiting gives to vegetative growth (Crowther, 1932-3).

In view of the difference in the length of day in the two countries at the time of the production of flower-buds, a possible effect of photoperiodism cannot be ignored. Cotton is a short-day plant, and when subjected to long days is liable to shed its flower-buds profusely. This has been demonstrated by Onodera (1938) in Korea by growing varieties from more southerly latitudes. Konstantinov (1930) found that flowering was most rapid in a 10-hour day and that length of day exerted most influence on the latest-ripening types, a shorter day causing much earlier sympodial development. Since the day in Egypt is of 14 hours' duration at the time of flowering, and is 2 hours longer than that in the Sudan, this longer daylight, it might be argued, could have contributed to the heavier shedding of flower-buds in Egypt. Yet, since the sympodial branches began at a lower main-stem node in Egypt, whereas the reverse would have been expected if branching in these countries were controlled by length of day, it seems unlikely, at least with the varieties examined, that photoperiodism played any important part in the differences between the Sudan and Egypt in the production and shedding of fruits.

#### FLOWERING CURVES

Since each cotton flower remains in bloom for a single day only, counts of the daily production of flowers are a simple measure of the rate of crop growth. Balls (1915) stressed the value of the flowering curve, and early reports in 1924 and the following years from the new experimental stations of the Empire Cotton Growing Corporation, included flowering curves in an application of Balls's methods to the solution of local physiological problems.

Data for 12 years from the Old observation plot grouped into weekly totals are presented for the Sudan in Fig. 3 (f), but the corresponding curve for Egypt is from a single experiment at Bahtim in 1934. This experiment was on land of high fertility, but although the peak of flowering was therefore abnormally high the general shape of the curve is typical of those obtained by others in Egypt, for example, Prescott (1924a) and Bailey and Trought

(1927). Allowing for this difference in fertility, the flowering curves of both countries closely reflect the plant diagram of Fig. 3 (e); the sharp rise and fall in the Egypt curve reflects the proportionately rapid rate of production of flower-buds per unit of plot, while the gradual rise and fall of the Sudan curve reflects the wider distribution of flower-buds up the scaffolding of the plant.

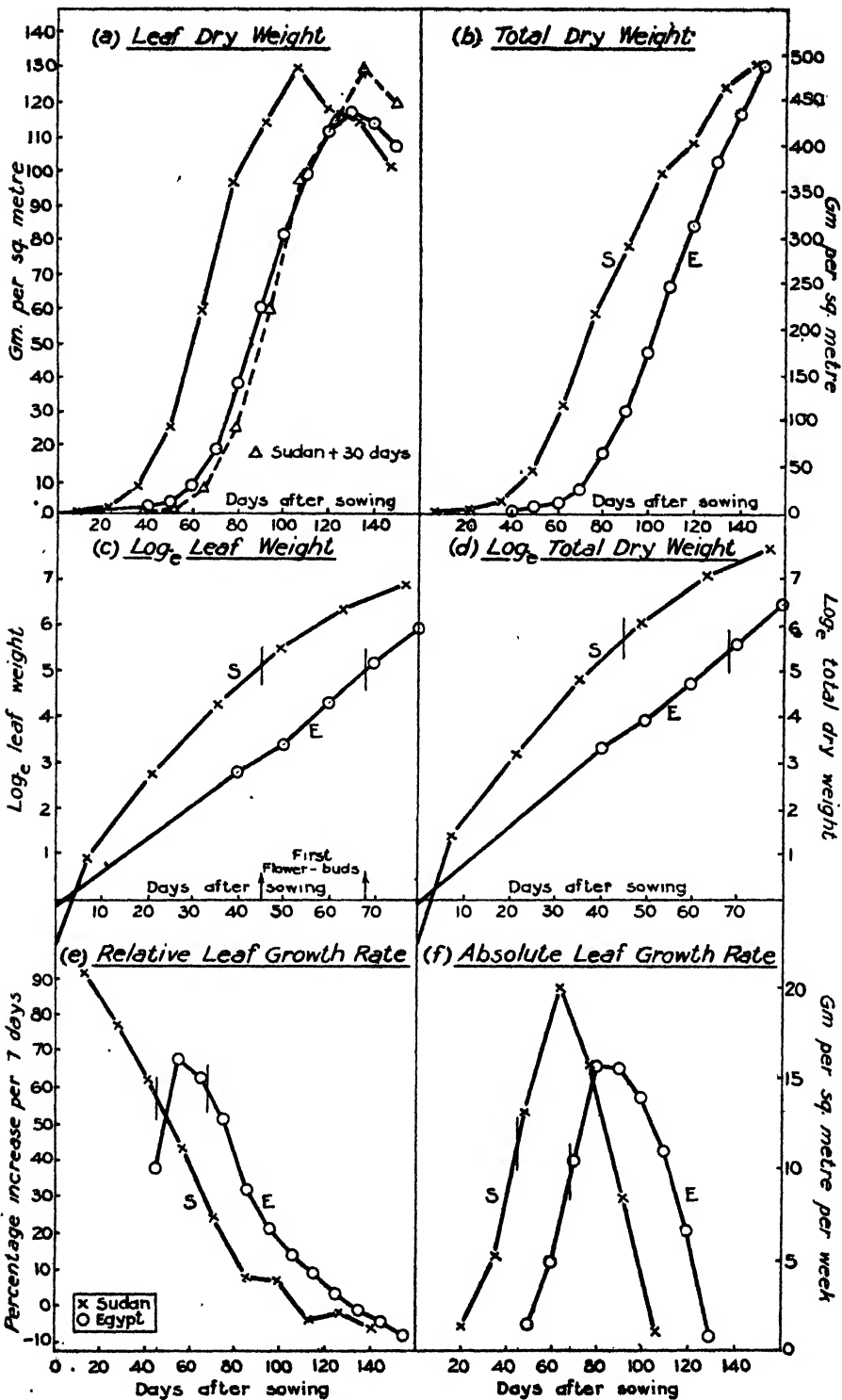
The Sudan plants at the beginning of flowering were 3 to 4 weeks younger than the Egypt ones, but the flowering period was prolonged for about that same length of time, so that both crops ceased flowering at a similar age. This delay of flowering in Egypt was commensurate with the delay of up to 4 weeks in node production shown in Fig. 2 (a), but it was to some extent offset by the initiation of flowering at a slightly lower node, as illustrated in Fig. 3 (e).

Prescott (1922) showed that the cumulative flowering curve of Egypt was characteristically sigmoidal and consequently that data from a large number of experiments could, by the use of logarithms, be expressed in linear form with considerable accuracy by an equation of the type

$$\log \frac{x}{a-x} = K(t-t_1),$$

where  $x$  is the number of flowers produced to time  $t$ ,  $K$  a constant,  $a$  the final number of flowers produced, and  $t_1$  the time when flowers produced reach a total of  $a/2$ . A typical curve of Prescott (1924a) based on his Egyptian data, is reproduced in Fig. 3 (g) for comparison with the curve from the Sudan calculated by the same method from the data plotted in Fig. 3 (f). The experimental values agree closely with the theoretical in both countries, and consequently the cumulative flowering curve in both is sigmoidal in form. It must be noted that the period of close agreement includes in both cases the *falling* curve after the peak. This is important in view of the emphasis placed by Balls (1915) on the curtailment of the flowering curve by root asphyxiation through a rise in the water-table. Templeton (1930) and Bailey and Trought (1927) subsequently questioned this interpretation. Whether or not in these years the check was caused by the rise in the water-table before damage by bollworm became severe, in recent years, in which measures have been adopted to ensure an early crop which will escape much of the damage, factors other than the water-table have curtailed the flowering curve. Of the 12 experiments made in Egypt by the author, only 2 showed any marked rise in the water-table before mid-August, by which time flowering in all experiments had long passed its peak (Crowther, 1937). As stated before, the Sudan Gezira has no water-table in the agricultural sense. Since the average flowering curves of both countries are of the same type, it may be concluded that the fall in both cases was determined by the same cause.

Balls (1917), as a result of a study of the flowering curve, put forward an hypothesis of 'predetermination' in which "The principal cause of the daily fluctuations in rate of flowering during the early part of the curve is to be traced to fluctuations in the rate of elongation of the main stem, about four weeks previously'. Although, subsequently, Bailey and Trought (1927) disagreed with this conclusion and explained the fluctuations in terms of flower-



bud shedding, the principle of predetermination is a firmly established one. In the present paper daily fluctuations are not discussed, but the shape of the flowering curves, based on weekly rates for both Egypt and the Sudan, illustrates the principle of predetermination. For seasons normal in weather and pest incidence the curve for weekly flowering, it is suggested, was predetermined by the earlier rate of production of leaf growth, the amount of which was itself closely associated with the rate of absorption of nitrogen from the soil, as will be shown later.

#### AMOUNT AND RATE OF PRODUCTION OF DRY MATTER

This investigation, up to the present, has been concerned mainly with the growth of the plant's framework and the production upon it of the potential crop. It has been shown that differences in temperature in the two countries produced a marked contrast in the rates of the early growth of the crops in the field, and differences in their spacing controlled their differing production and development of fruiting branches. These fruiting branches are numerous in both countries and morphologically are capable of greater development than appeared from these experiments. The reasons for the limitation of their growth should be elucidated by an examination of the amount and efficiency of foliar development.

The methods used in obtaining dry weights and calculating growth rates have already been described for the Sudan (Crowther, 1934) and for Egypt (Crowther, 1937). Random samples of plants cut off at ground level were collected and separated into leaves, stems, flower-buds, and bolls. The total oven-dry weights therefore represent those for the aerial portions of the plant only. The basis for dry weights and nitrogen content is not the single plant but a unit area of field. For the Sudan the data cover a period of 11 years on the Old observation plot, and for Egypt 10 experiments made during 2 seasons. ✓ The dry weights of *leaf* and of *the whole plant* are given in Figs. 4 (a) and 4 (b) respectively. Leaf weights reached a maximum in the Sudan at 105 days after sowing, and in Egypt at 130 days. This delay of  $3\frac{1}{2}$  weeks reflected, as in the case of the data for heights and nodes, the limitation of growth in Egypt by low temperatures. Weights of total dry matter showed a similar contrast until 100 days, after which the increase in the Sudan was smaller, while in Egypt dry weight continued to increase rapidly up to the last sample. When allowance had been made in the Sudan data for the effect of manuring there was little difference between the two countries in either maximum leaf weight or total dry weight attained, on a basis of unit area of field; although, allowing for the spacing differences, the individual plant in the Sudan produced more than twice the leaf growth of that in Egypt.

Heath (1937b) found that the dry weight of rain-grown cotton in South Africa, as well as the height of the main stem, increased exponentially from

FIG. 4. Graphs showing dry weights of leaf and whole plant (aerial parts) and growth rates. Sudan curves averaged from 11 years' data, Egypt curves from 10 experiments made during 2 years.

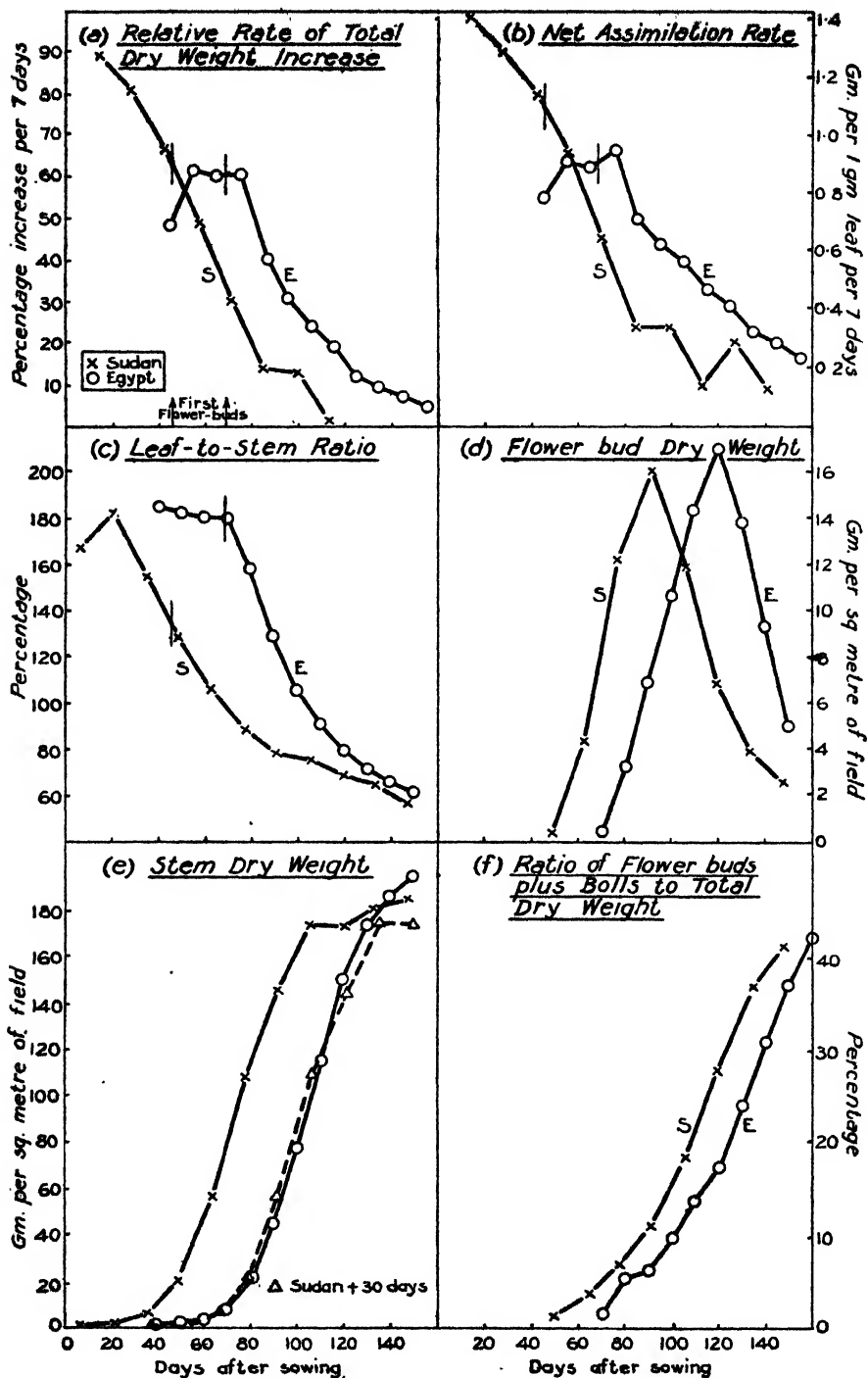


FIG. 5. Graphs showing growth rates, dry weights, and ratio of dry weights. Sudan curves averaged from 11 years' data, Egypt curves from 10 experiments made during 2 years.

shortly after germination until flowering. Figs. 4 (c) and 4 (d) express the logarithms of the dry weights of leaf and whole plant, and Fig. 4 (e) and Figs. 5 (a) and 5 (b) show respectively the relative leaf growth rate, efficiency index (relative rate of total dry-weight increase), and net assimilation rate calculated from the same data. The increases in leaf weight and in total dry weight for Egypt appear from Figs. 4 (c) and 4 (d) to be approximately exponential until flowering, yet, as Fig. 4 (e) shows, the relative growth rate was never in fact constant for any considerable period, but increased from the earliest sample to a maximum 8 weeks after sowing. In the Sudan relative growth rates for both leaf and total dry weight fell progressively from the earliest sample until the time of maximum leaf weight, with no indication of a maximum growth rate at 8 weeks after sowing such as occurred in Egypt.

In the Sudan net assimilation rate, as shown in Fig. 5 (b), was higher during the first month after sowing than at any time in Egypt, and in both countries the subsequent changes in net assimilation rate with time resembled those in relative leaf-growth rate, except that the former declined less rapidly. Heath (1937a) found up to the time of the flowering no general rise and fall in net assimilation rate, measured as here on a basis of leaf weight. In this respect the Sudan crop differed markedly from the rain-grown cotton of South Africa.

A possible explanation may lie in differing air humidities. Water supply is known to influence cell extension in the internodes of the main stem and may exert a similar effect on the individual cells of the leaves, thereby altering leaf area. It has been found (Crowther, 1933-4) that date of sowing in the Sudan affected the thickness and area of leaves, so that late sowings, which were subjected to high temperatures and high saturation deficit, had smaller, thicker leaves than earlier sowings made during the rains when temperatures and saturation deficit were lower. On this basis the leaves in Egypt would, in general, be expected to have a lower weight per unit area than those in the Sudan, and net assimilation rate calculated on a basis of leaf weight would, except for the period of early growth during the rains, be lower in the Sudan than either in Egypt or in South Africa where rain fell at intervals throughout growth. This hypothesis would explain the differences in Fig. 5 (b), but from existing data it is impossible to decide whether the effect is primarily one of humidity or merely of high temperature.

Comparison of carbon assimilation in the two countries must include some reference to the conflicting results on stomatal movements observed between the Sudan and Egypt. Balls (1912), in Egypt, found that stomata not only closed at night, but that after opening in the early morning they closed *before* the evening. He stated: 'On hot, dry, windy days, with a soil approaching to physiological dryness, the full aperture has scarcely been attained before closure sets in. . . . By noon, on most summer days, this closure is almost complete.' In the Sudan, despite the more desiccating conditions, Crowther (1933-4) found no indication of midday closure. Experiments made on 22 days at the hottest time of year showed that on 18 days there was rapid opening from daybreak, with maximum aperture at noon, followed by gradual closing

until almost sunset, when complete closure followed rapidly; these included a day when the maximum shade temperature was  $42^{\circ}\text{C}$ . On the other 4 days partial closure occurred whenever the sun was obscured by cloud. That the apertures are wide open during daytime in the Sudan has also been found recently by Barlow (unpublished work). Novikov (1930) found in Russia the stomata of cotton widest open between the hours of noon and 1 p.m., closure occurring gradually after 3 p.m. They were at maximum aperture in brightest sunshine and less in cloudy weather. These findings agree closely with those of the Sudan. This contrast between the two countries calls for re-examination, since it is important in its bearing upon the factors which limit growth. The midday closure found in Egypt has rendered strong support to interpretations of limitation of growth in terms of 'water-strain', whereas in the Sudan the absence of midday closure has been evidence for the view that air humidity as a factor limiting growth and causing shedding is unimportant in comparison with some factor, either internal or in the soil, such as nitrogen supply.

When the leaf data were expressed as rate of *absolute* increase in dry weight per unit area of plot, as in Fig. 4 (f), instead of on a relative basis, it was seen that the rapid early growth of the Sudan plants enabled them to produce a given leaf weight  $3\frac{1}{2}$  weeks earlier than those in Egypt. The rate ultimately fell off earlier in the Sudan by a similar period, so that the general shape of the two curves was similar. In both countries the maximum rate of production of leaf weight per unit area followed 3 weeks after the appearance of the first flower-buds. That interval approximates to the time that Bailey and Trought (1927) found was taken by the flower-bud to pass from the 'plainly visible' to the 'open flower' stage. Thus the maximum rate of leaf-weight production in both countries coincided with onset of open flowering, and from that point the rate fell rapidly. By comparing Fig. 4 (f), showing the absolute leaf-growth rate, with Fig. 5 (d), which shows the dry weight of total flower-buds at successive stages, it is seen that this rapid fall in leaf-weight production was followed 30 days later by a similar fall in flower production. Indeed the whole cycle of flower production in both countries resembled that of leaf-weight production 30 days earlier, the only difference being that the plants in Egypt produced slightly more flower-buds per unit of leaf weight. This, it is suggested, furnishes an example of 'predetermination', the factor determining the curtailment of flower production being the decline in the rate of leaf growth 30 days previously. This decline itself arose from the onset of the reproductive phase through diversion of assimilated material from the leaves to the flower-bud initials (Crowther, 1934).

It has been stated above that the relative leaf-growth rate in Egypt fell away after about 8 weeks because of the demands of the flower-buds. Yet in the Sudan, though the flower-buds appeared 45 days after sowing, the relative leaf-growth rate began to decline at least a month before that time. The reason for this early decline is obscure, but supra-optimal temperatures, which Gregory (1928) found reduced the leaf-growth rate of *Cucumis* as noted in the discussion of the height data, may be in some degree responsible, especially

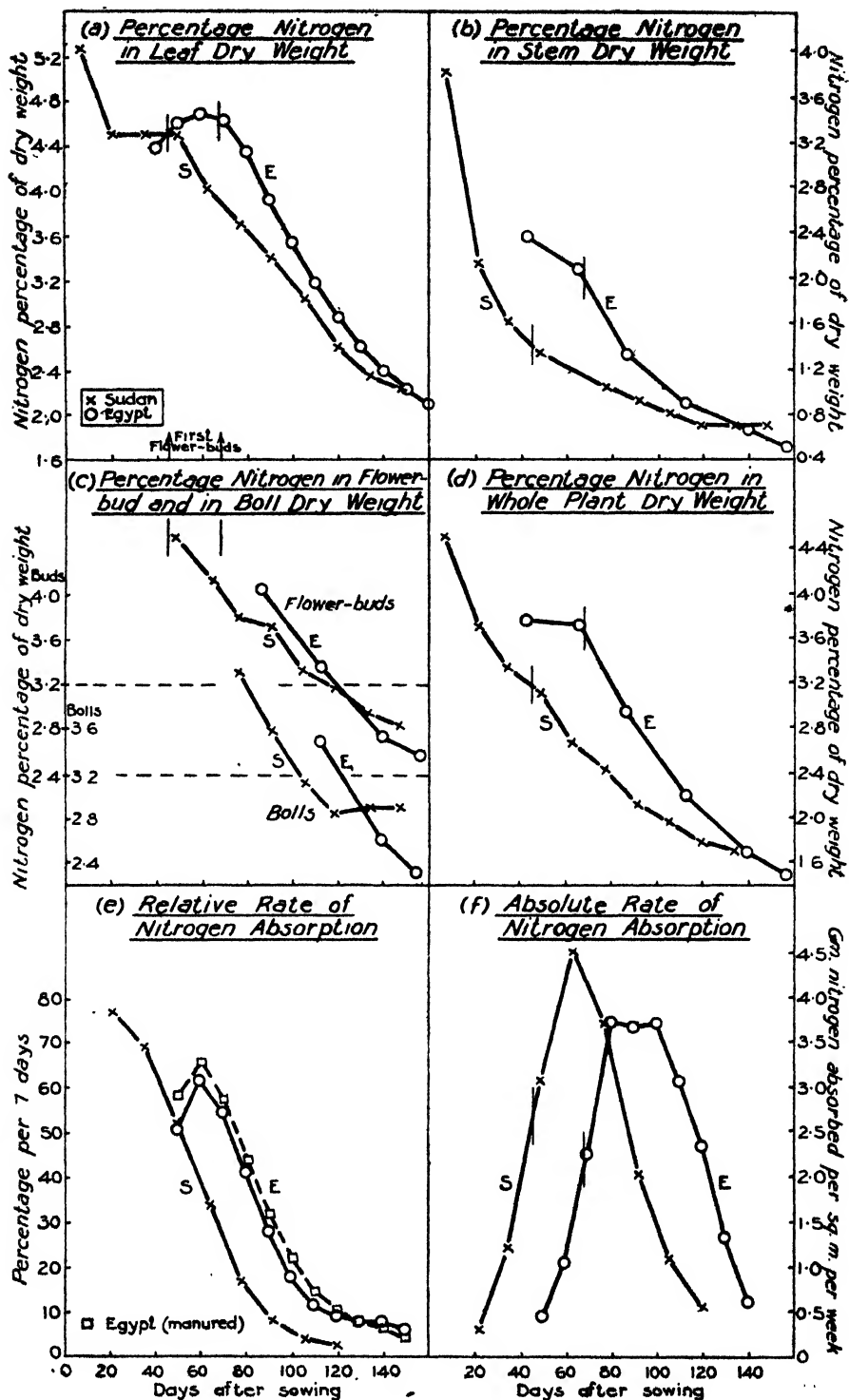
since Gregory found that at such temperatures more growth was made relatively by the stem than by the leaves. In the Sudan, after sowing, maximum temperatures become increasingly above optimum until 2 months after sowing, and, as for Cucumis, there was a reduction in the ratio of leaf to stem dry weight in the Sudan, as shown in Fig. 5 (c). The ratio fell progressively after a maximum at 3 weeks, whereas in Egypt, where temperatures were not supra-optimal, it remained constant until the formation of flower-buds.

The data for dry weights of *stems*, plotted in Fig. 5 (e), show that their rate of increase, like the corresponding rates for leaf and total dry weight, was higher in the Sudan than in Egypt during the first 2 months after sowing. During the third month the rates of stem growth in the two countries were similar, but since subsequently in the Sudan the rate fell off sharply, the total weights of stem per unit area of field at the end of the season were similar in both countries.

A third curve, included in Fig. 5 (e), shows the dry weight of stem in the Sudan, with a shift of 30 days along the time axis—a period chosen to correspond with the delay occasioned by low temperatures in Egypt. As a result of the shift, the curve of the Sudan lies close to that of Egypt throughout the main growth period. Thus low temperatures in Egypt depressed the rate of increase in stem weight at the time, but were without after-effects on later growth. In Fig. 4 (a), when a similar time shift was made for the data of leaf dry weight, the values for the Sudan fell behind those for Egypt until near the time of maximum leaf weight; whereas for the stem data the slight difference between the curves indicated that the Sudan was ahead of Egypt. Hence the early fall in leaf-to-stem ratio in the Sudan arose from a failure by the leaves, in contrast with the stems, to maintain as high a rate of growth as in Egypt for the same stage of morphological development. In fact, growth was approximately exponential in Egypt for both stems and leaves but in the Sudan for stems only. Since most of the stem dry weight is of main stem this conclusion is surprising, for the height data showed that in the Sudan the main stem *failed* to maintain an exponential rate of elongation. In terms of the hypothesis suggested for the leaf data it appears that temperatures which were supra-optimal for rates of leaf-weight increase and stem elongation were not so for the rate of translocation of carbohydrate from the leaves. In this way the stem in the Sudan was an increasing drain on assimilated products, just as on a larger scale and at a later stage of the plant's development were the flower-buds in both countries. This will be discussed later, after the examination of the analyses of plant nitrogen given in the next section.

With the formation of flower-buds and their subsequent development, the proportion of dry weight accumulated in the *flower-buds and bolls* increased rapidly. The dry weights are included in Fig. 5 (d) and the proportion of buds plus bolls in total dry weight in Fig. 5 (f). In the Sudan the proportion reached 40 per cent. at 20 weeks after sowing, whereas in Egypt the same proportion was not reached until 16 days later. This delay again reflected the initial retardation of growth in Egypt by low temperatures, partially offset by the





closer spacing, as described in a previous section. That such a high proportion of the total dry matter should, in both countries, eventually be present in buds and bolls emphasizes the severity of the drain exerted by the developing fruits.

#### ABSORPTION OF NITROGEN

##### (a) *Percentage of nitrogen in dry weights.*

The nitrogen content of the separate aerial parts of the plant, namely leaf, stem, buds, and bolls and of the whole plant, expressed as percentages of dry weight and averaged from 10 seasons' data for the Sudan and from 10 experiments made during 2 seasons for Egypt, are plotted in Figs. 6 (a) to 6 (d).

In Egypt the percentage of nitrogen *in the leaves* rose to a maximum 60 days after sowing and thereafter fell away rapidly to minimum values at harvest. In the Sudan the first sample had the highest value, and the last the lowest. This Sudan curve was not affected by the application of ammonium sulphate 6 weeks after sowing, except that the final values at harvest may have been raised slightly by manuring (cf. Crowther, 1934). Since sampling in Egypt did not begin until 40 days after sowing, it is impossible to compare the plants of the two countries in detail before that date. From knowledge of the nitrogen content of cotton seed in Egypt and of the ratio of the weights of cotyledon to seed coat, it can be assumed that the cotyledons at sowing had a value of at least 5 per cent. Therefore in Egypt, as in the Sudan, there was first a diminution in percentage following germination; then in the Sudan the percentage remained constant until flower-bud formation, whereas in Egypt the percentage increased to a second maximum. At the time of the appearance of flower-buds the percentages of nitrogen in the leaves were similar, i.e. 4.5 per cent. in the Sudan and 4.6 per cent. in Egypt; subsequently they both fell steeply to low values of 2.0 and 2.2 per cent. respectively at the time of the opening of the bolls.

Fig. 6 (b) shows that for both countries the percentage of nitrogen *in the stems* was highest in the earliest sample and declined progressively to minimal values at harvest, without indication of the second maximum reflected in the data for leaf nitrogen. Throughout the main period of growth the stems in Egypt had consistently the higher percentage of nitrogen. Even at the time of the formation of flower-buds, when the values in the leaves were similar, those for the stems were 1.9 per cent. for Egypt and 1.5 per cent. for the Sudan.

The early values for percentage of nitrogen *in the flower-buds and bolls*, shown in Fig. 6 (c) for the two countries at corresponding stages of development, were similar. The progressive diminution with time reflected the increase in average age of the samples of fruit, for the percentage of nitrogen was highest in the young bud and lowest in the mature boll. The fall in the

FIG. 6. Graphs showing nitrogen content as percentage of dry weight in the separate parts of the plants, also rates of nitrogen absorption. Sudan curves averaged from 10 years' data and Egypt curves from 10 experiments made during 2 years.

percentage was more rapid in Egypt than in the Sudan, a consequence of the more rapid rate of fruiting per unit area, arising from the closer spacing.

The change in the percentage of nitrogen in the entire aerial portion of the plants, shown in Fig. 6 (*d*), in both countries resembled that of the stems. During the first 2 months the fall was the more rapid in the Sudan so that, at the time of formation of flower-buds, the Sudan plants had the lower percentage. Subsequently the decline was the more rapid in Egypt until at harvest the percentage was equally low in both countries.

Recently Parnell and MacDonald (1942) published results of the nitrogen analyses of cotton at Barberton, South Africa, and concluded: 'These results are clearly very different from those obtained by Crowther (1934) in the Sudan and it appears that his general nitrogen level is very much lower than ours.' Parnell and MacDonald sampled only fully formed young leaves, in contrast to Crowther, whose method in the paper quoted and in the present paper, dealt with the entire foliar system on the plants at the time of the sample. Portsmouth (1937) showed that the percentage of nitrogen in individual cotton leaves declines with increasing age, and no agreement should therefore be expected between the two sets of leaf analyses. Parnell and MacDonald recognized that the leaf samples were not strictly comparable but found their conclusions supported by the data for flower-buds. These, again, were not based on samples of buds of all ages but on buds due to flower the following day. Thus their values of 4.09 to 3.61 per cent. should correspond to the samples at later dates in the Sudan, as shown in Fig. 6 (*c*), and from these it is clear that the South Africa values for percentage nitrogen were indeed higher than those of the Sudan and Egypt. The lower final percentages for flower-buds in Egypt, as compared with the Sudan, were ascribed above to the close spacing of the Egypt plants, which led to acute 'internal starvation'. Presumably the spacing in the experiments of Parnell and MacDonald was similar to that of Heath at Barberton (1937), namely 2 ft. by 3½ ft., equivalent to 1.5 plants per sq. metre as compared with 5 plants and 13.3 plants respectively for the Sudan and Egypt. It is concluded that the South Africa data would in any case show higher values for nitrogen in the flower-buds because the spacing there was wider than in the Sudan. How far these higher values are the result of spacing or of soil differences is not evident from existing information. That the nitrogen supply was adequate with the existing spacing at Barberton was indicated by the absence of response to nitrogenous fertilizer, in marked contrast with the response found in the Sudan and Egypt.

(*b*) *Rate and amount of nitrogen absorption from the soil.*

The relative and absolute rates of absorption of nitrogen by the whole plant (aerial parts) in both countries are given in Figs. 6 (*e*) and 6 (*f*). The curves for *relative rate* show the same trend with time, and the same differences between the countries as those for relative leaf-growth rate.

The Sudan plant shortly after germination absorbed nitrogen more rapidly than the Egypt plant at any stage of growth, relative to their respective sizes.

In Egypt the most rapid rate of absorption, relative to the size of the plant, was at 60 days after sowing, when the rate of root growth had accelerated, following considerable increase in temperature, while leaf growth was as yet unchecked by the demands of the developing flower-buds. As the Sudan curve was based on manured cotton, a similar curve for Egypt is included in Fig. 6 (e) to show that the difference between the two countries in rate of absorption of nitrogen throughout the season is but little affected by added nitrogen.

When allowance was made for the retardation in early growth by low temperatures in Egypt, the curves of both countries for *absolute rate* of absorption of nitrogen, presented in Fig. 6 (f), corresponded closely, the Egypt curve in effect repeating the Sudan one after an interval of 26 days. The maximum rate of absorption in both countries coincided with the initiation of flowering, and the onset of fruiting is regarded as responsible for the subsequent fall (Crowther, 1934). In both cases nitrogen absorption fell to low levels at the time of boll opening, through, it is suggested, a check to root growth from 'internal starvation'.

The integration of these varying rates of absorption is reflected in the data for the *total nitrogen absorbed by the crop* in each country, presented in Table IV.

TABLE IV

*Amounts of Nitrogen (kg. per feddan) absorbed by the Crop in the Experiments (aerial portions only)*

| Sudan.                   |        | Egypt.    |              |
|--------------------------|--------|-----------|--------------|
| Unmanured New obs. plots | . 27.7 | Unmanured | . . . . 34.7 |
| Manured Old obs. plot    | . 30.3 | Manured   | . . . . 44.3 |

For the Sudan the 10 years' data of the Old observation plot, which has supplied most of the growth data presented in this paper, show an average 30.3 kg. nitrogen per feddan while the 10 experiments which supplied the developmental data for Egypt averaged 34.7 kg. nitrogen per feddan, representing a removal of nitrogen 14 per cent. greater in Egypt than in the Sudan. Analyses of cotton in Egypt by Foaden and Mackenzie (1899) indicated a removal of 27 kg. nitrogen by the cotton crop of that time, and the higher value shown in Table IV may be ascribed to the close spacing now general in Egypt. For the Sudan, Joseph's analyses (1920) gave a removal by unmanured cotton of 27.2 kg. per feddan, with which the appropriate value in Table IV closely agrees. The unmanured and manured values for the Sudan cannot be compared as reflecting the extent of recovery of added nitrogen, since they cover a different range of seasons. The separate seasons of the Old observation plot, which together averaged 30.3 kg., included two when the recovery was below 11 kg. and three when the recovery exceeded 40 kg.

(c) *Effect of nitrogen absorption upon the development of the plant.*

At this point it is illuminating to re-examine the growth data in the light of the nitrogen analyses, by comparison of the relevant figures.

*Nitrogen content and growth of leaves.* There is a striking resemblance between the curves for percentage nitrogen in the leaves, shown in Fig. 6 (a), and those for relative leaf-growth rate shown in Fig. 4 (e). In both cases there was a maximum in the data for Egypt at 60 days after sowing, and in both the values for the Sudan were highest at the first sample. The only discrepancy was in the Sudan curves at 20 to 50 days after sowing, for whereas during that time the growth rate declined progressively the percentage nitrogen in the leaves remained constant. Subsequently the fall was resumed, and in both countries when the leaves ceased growing the percentage of nitrogen was about 2.4 per cent.

Gregory (1926), working with barley in sand culture, found that differences in relative leaf-growth rate could be explained in terms of the nitrogen factor and concluded: 'Relative leaf growth rate is largely dependent on internal factors and is relatively independent of external conditions; whereas, in contrast with this, net assimilation rate has been shown to be wholly controlled by external factors.' With cotton, Crowther (1934) found in the Sudan Gezira a highly significant effect of added nitrogen on relative leaf-growth rate, and the data led Gregory (1934) to conclude, 'Leaf growth rate is conditioned almost entirely by the rate of supply of nitrogen'. The closeness of this relationship is illustrated in Fig. 7 (a), where relative leaf-growth rate is plotted against the corresponding value for percentage leaf nitrogen at successive samples. The period covered was the entire development from the earliest stage until boll maturation. Some correlation is inevitable between a falling growth rate and the percentage content of nitrogen or of any mineral constituent, which must necessarily fall with time through dilution by carbohydrates as the plant ages. Yet in view of these earlier findings of Gregory and Crowther, and of the closeness of the agreement between the Sudan and Egypt, it is concluded that relative leaf-growth rate in both countries was primarily controlled by the degree of concentration of nitrogen in the leaves. As a result of the correlation a determination of the percentage of nitrogen in the total green leaves, irrespective of the age of the plant, indicates how rapidly the crop is growing.

The following are the regression equations for parabolas of closest fit for each country:

$$\text{Sudan } y = 1.568 - 1.205x + 0.225x^2,$$

$$\text{Egypt } y = 0.235 - 0.304x + 0.082x^2,$$

where  $y$  is the relative leaf-growth rate and  $x$  the percentage nitrogen of leaf dry matter. These equations are shown graphically in Fig. 7 (a). Of the variance in growth rate in Egypt 98.7 per cent. could be attributed to change in percentage leaf nitrogen, and in the Sudan 93.7 per cent. The lesser degree of agreement in the Sudan arose from the period of storage of nitrogen in the leaves described below, the only period over which in either country the above-mentioned correlation did not hold.

A detailed account of the changes in the percentage nitrogen with time is

not possible here. Briefly it can be stated that the high values at field germination in the Sudan, shown in Fig. 6 (*a*), represented the accumulation of nitrogen in the seed, for in the seed at sowing two-thirds of the nitrogen present is in the cotyledons. The sharp fall in the percentage was associated with the migration of nitrogen into the stem and radicle, and with dilution through carbon assimilation. The fall was checked about 20 days after sowing by absorption of nitrogen by the roots which were by then well established. In the Sudan Gezira this fall was more marked in some seasons than in others, giving rise to a correlation between the percentage leaf nitrogen shortly after sowing the final yield (Lambert, 1936-7; Crowther, 1941).

It has already been pointed out (Crowther, 1941) that this correlation measures neither the differing percentages of nitrogen in the seed sown each season nor the especial importance of nitrogen supply in the seedling stage. It is an index of soil conditions, reflecting seasonal differences in the total amount of nitrogen available to the crop. Uptake of nitrogen being proportional to external concentration (Gregory, 1937), a low value for percentage nitrogen indicates a season of low nitrogen supply. After the fall, taking the average of 10 seasons, the percentage remained constant for 30 days, i.e. until the formation of flower-buds was well under way. This was the time when the nitrogen was stored in the leaves, there being an excess over the amount needed for the rate at which leaf growth was proceeding; possibly at this stage this rate was itself controlled by supra-optimal temperatures as already mentioned. Because of this storage, as will be seen from Fig. 7 (*a*), there was in the Sudan a wide range of relative leaf-growth rates for the maximum nitrogen content of 4.5 per cent.; this renders an estimate of growth rate on a basis of leaf nitrogen, at this particular phase of development, liable to large error. Storage of nitrogen in the leaf ceased abruptly at the onset of fruiting, and from this stage onwards the relative leaf-growth rate was no longer governed by external factors but by the amount of nitrogen which remained behind in the leaves.

In Egypt, as in the Sudan Gezira, there was also a drop after germination in the percentage nitrogen in the leaf. Subsequent recovery, by comparison, was retarded, though the same maximum of 4.5 to 4.6 per cent. was eventually attained in both. During the period of the 25 days prior to the formation of flower-buds, i.e. 45 to 70 days after sowing, there was a marked increase in relative leaf-growth rate to a maximum at 55 days, coincident with an increase in percentage nitrogen in the leaves. During that period net assimilation rate, which is governed primarily by external factors, was constant. Thus it appears likely that in Egypt, even despite the very low temperatures, relative leaf-growth rate was controlled primarily by leaf nitrogen supply. This agrees with Gregory's conclusion (1928) that at sub-optimal temperatures relative leaf-growth rate is independent of temperature. Presumably low temperatures limited root growth and the rate of absorption of nitrogen. The increase in night temperatures during April would be followed by more rapid root development and consequently by more rapid uptake of nitrogen.

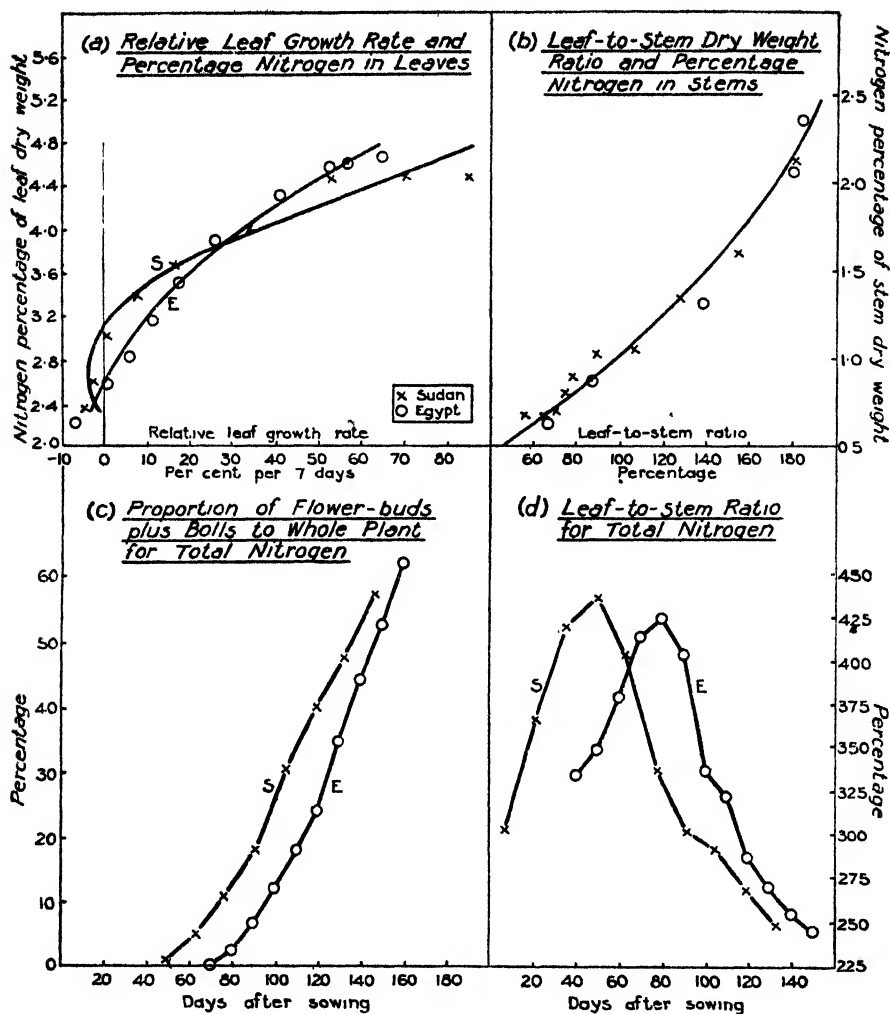


FIG. 7. Graphs showing (a) successive determinations of relative leaf-growth rate plotted against corresponding values for percentage nitrogen in leaf dry weight, (b) successive determinations of leaf-to-stem ratio for dry weight plotted against corresponding values for percentage nitrogen in stem dry weight, (c) distribution of nitrogen between fruits and vegetative parts, viz. the amount of total nitrogen present in flower-buds and bolls expressed as a percentage of that present in the whole plant (aerial parts) and (d) amount of total nitrogen present in leaves expressed as a percentage of that in stems. All curves are derived from data presented in Figs. 4-6.

This may explain the remarkable results of dibble sowing in Egypt (Gracie and Balls, 1939), where large increases in final yield resulted from sowing fewer seeds with a special dibble and covering with sand instead of with parent soil. Fewer seedlings were less affected by competition for nitrogen supply than under the old method of sowing ten or more seeds per hole. Moreover, Gracie and Balls found that small-seeded varieties profited more

from dibble-sowing than larger-seeded ones, and they ascribed this to the difficulty with which the small seeds 'break their way out'. In terms of nitrogen large-seeded varieties may be expected to have the initial advantage of more nitrogen in the cotyledons; small-seeded varieties would therefore tend to benefit the more from dibble-sowing if it led to increased absorption of nitrogen. In the Sudan, where early root growth is rapid, dibble-sowing was noticeably without effect on growth and yield (Crowther, 1940-1).

*Distribution of nitrogen and dry weight between leaf and stem.* In the Sudan there was no indication in the stems of the interruption in the falling percentage of nitrogen found in the leaves. Evidently the storage of nitrogen in the leaves immediately prior to the formation of flower-buds was not supplemented by similar storage in the stems, which confirms earlier findings (Crowther, 1934). Fig. 7 (*d*) gives the ratio of total nitrogen in the leaves to total nitrogen in the stems. Here the curves of the two countries are of similar shape, in contrast with those for the same ratio expressed in terms of dry matter, as in Fig. 5 (*c*). In the Sudan the premature fall in the ratio expressed in terms of dry matter arose when the dry weight of leaves failed to increase as rapidly as that of the stems, but since *percentage* nitrogen remained constant in the leaves by reason of the nitrogen stored in them, while still falling in the stems, the *total* nitrogen in leaves and stems showed a rising ratio over the same period, which was similar to that following about 30 days later in Egypt. There the leaf-to-stem ratio for dry weights remained constant during the period of restricted early growth, while the percentage nitrogen in the leaves rose to a second maximum at 60 days and in the stems declined, giving a rising leaf-to-stem ratio for total nitrogen. In both countries the maximum in this ratio was reached at the initiation of fruiting, the nitrogen in the leaves thereafter migrating rapidly to the flower-buds until, as shown in Fig. 7 (*c*), at least 60 per cent. of the nitrogen in the entire aerial parts of the plant was concentrated in the developing fruits at harvest, while that in the stem remained constant (Crowther, 1934).

Regarding the stems, those in the Sudan may be seen from Fig. 6 (*b*) to have had lower values for percentage nitrogen than those in Egypt at corresponding stages of development. The change with time and the contrast between the Sudan and Egypt were both suggestive of the corresponding curves for the ratio of leaf dry weight to stem dry weight given in Fig. 5 (*c*). The degree of correlation between them is evident from Fig. 7 (*b*). Despite the apparent wide divergence between this ratio in the two countries it can be estimated from a determination of the nitrogen in the dry matter of the stems whether the crop is growing in Egypt or the Sudan, whatever its age up to the time of boll-opening. The quadratic equation of closest fit, considering both countries simultaneously, is

$$y = -14.55 + 132.0x - 19.06x^2,$$

where  $y$  is leaf weight as percentage of stem weight, and  $x$  the nitrogen percentage of stem dry matter. From this equation 97.3 per cent. of the



variance of the values for leaf-to-stem ratio shown in Fig. 5 (c) can be associated with change in the nitrogen percentage of the stem.

This relationship may be examined on a basis of the conclusions reached regarding changes with time in dry weight and nitrogen composition. In both countries the rate of increase in stem dry weight proceeded exponentially for several weeks, despite high temperatures in the Sudan which appeared to cause there a fall in the relative rate of leaf growth. This relation between percentage nitrogen in the stem and the ratio of leaf to stem dry weight indicates that the falling relative leaf-growth rate must have been accompanied by a fall in the translocation of nitrogen, relative to carbohydrates, from the leaves into the stems. This is in agreement with a suggestion which Gregory (1934) advanced to explain the restricted development of late sown plants in the Gezira in terms of changed internal nitrogen metabolism caused by high temperatures. In Egypt, where growth was restricted in the early stages by low temperatures and the plants remained 'juvenile', there was little migration of carbohydrates to the stems; the ratio of leaf to stem dry weight was unaffected and the nitrogen in the stems suffered little dilution.

Since the relation between percentage nitrogen in the stem and the leaf-stem ratio holds not only for high temperatures but over the whole range of conditions experienced by the crops in both the Sudan and Egypt, the rate of transport of nitrogen from leaf to stem is evidently controlled primarily by the leaves, whereas the similar movement of carbohydrates is determined primarily by the demands of the stems. Thus the stems with their accumulation of carbohydrates in mechanical tissues contrast with the fruits which, by reason of their meristematic activity, accumulate nitrogen at the expense of the leaves. Hence it appears that, whereas during early growth percentage nitrogen in the leaves is an index of external nitrogen supply, percentage nitrogen in the stems reflects the internal distribution of carbohydrates and nitrogen between the separate parts of the plant.

#### YIELDS OF THE CROPS

The yields of the experiments are summarized in Table V.

TABLE V  
*Yields of Seed Cotton in the Experiments*  
(Kantars per feddan)

| Sudan (Sakel only).        |      | Egypt.                   |      |
|----------------------------|------|--------------------------|------|
| Old obs. plot (manured)    | 5.44 | Average of all varieties | 5.41 |
| New obs. plots (unmanured) | 4.60 | Sakel only (unmanured)   | 3.99 |
|                            |      | „ „ (manured)            | 4.46 |

The Sudan data are derived from 13 seasons for the Old and from 3 seasons for the New observation plots. In Egypt the data for the average of all varieties are derived from 10 experiments made in 2 seasons, and those for Sakel only from 3 experiments. In the case of Egypt comparison of the

yields of manured and unmanured crops shows directly the effect of manure, but for the Sudan the two yields are not comparable, being obtained from different sets of seasons.

If a comparison of the two countries was limited to the Sakel variety only one would conclude from Table V that yields in Egypt were much below those of the Sudan. Such a conclusion is not supported by data from the commercial crops. Aboul Ela (1932) gave the average Sakel yield for Egypt as 3.25 kantars per feddan. In the commercial crop of the Sudan the average Sakel yield over the 13 seasons was 3.18 kantars and was therefore similar to Egypt. But these yields cannot be considered representative of the whole areas concerned, for in recent years the Sakel variety has been grown chiefly in the northern districts of both the Gezira scheme and the Nile Delta, and the soil of these northern areas is in both cases inferior to the rest.

Disregarding varietal differences, the average yield of the commercial crop for the entire area, over the 9 seasons 1930-8 inclusive, was 3.45 kantars in the Sudan Gezira, while that of the Egyptian Delta, over the same period, has been estimated from data given in the 'Egyptian Cotton Year Book, 1938-9', at 3.90 kantars, i.e. slightly higher than that of the Gezira.

In the experiments which have provided most of the developmental data utilized in the present comparison, where for Egypt several varieties were averaged, the experimental yields in the two countries were similar, namely 5.44 kantars for the Sudan and 5.41 kantars for Egypt. That these yields are 40 to 50 per cent. above those of the commercial crop is chiefly attributable to the greater care expended on the small areas involved.

#### BOLL CHARACTERS

Although the difference in the final yield in the two countries was not great, the rates of crop maturation differed widely, as is seen in the graph of amounts gathered at successive pickings, presented in Fig. 8. The curve for the Sudan is based on data for 13 years, that for Egypt on 10 experiments comprising different varieties.

A detailed comparison of the rates of boll-opening from the data here presented is impossible because of the differing intervals between successive pickings in the two countries and of the small number of pickings taken in Egypt. The picking season in the Sudan extended over  $3\frac{1}{2}$  months, while between the first and last picking in Egypt the interval was  $1\frac{1}{2}$  months only. The difference was partly a result of the closer spacing in Egypt, because, as the flowering curves in Fig. 3 (f) show, the bolls there, being carried within a narrow zone of main stem nodes on a large number of plants, opened during a short space of time; this is in contrast with the longer period taken in the Sudan where the crop was distributed over more main stem nodes.

When the bolls began to open the plants of the Sudan were younger than those of Egypt, and the difference in age at harvest was widened by the practice current in Egypt of letting the open bolls accumulate on the plant

to ensure a very heavy first picking. In the Sudan such accumulation is impossible since with the low humidity the bolls open more fully and strong winds blow the cotton to the ground unless there are fortnightly pickings.

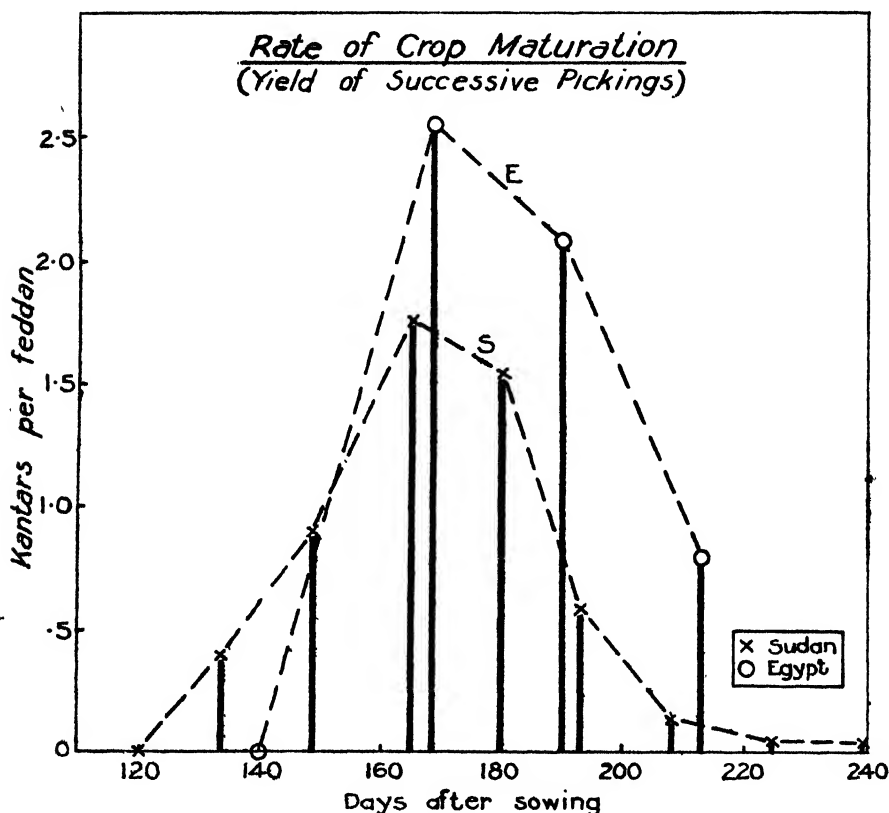


FIG. 8. Graphs showing rate of crop maturation expressed as yields of successive pickings of seed-cotton. Sudan yields averaged from 13 years' data, and Egypt yields from 10 experiments made during 2 years. (The curves are not directly comparable since the interval between successive pickings differed in the two countries.)

This renders 7 or 8 pickings necessary in the Sudan Gezira, whereas 2 or rarely 3 pickings suffice in Egypt.

Since variety may exert a marked influence on seed weight and lint characters, a comparison between the crops of the two countries becomes confused if several varieties are involved. Hence the following data for boll characters are confined to Sakel and this limits the Egypt data to the results of 3 experiments.

The maturation period is assessed as the number of days from the open flower to the open boll, and is obtained by labelling a random selection of flowers at weekly intervals, all bolls prematurely shed or diseased being excluded from the average. In Table VI average values for the Sudan

experiments are presented together with results quoted from other authorities since similar counts for the Egypt experiments were not available.

TABLE VI

*Average Maturation Period of Bolls (days)*

| Sudan.                |   |    | Egypt.                    |   |          |
|-----------------------|---|----|---------------------------|---|----------|
| Lambert (1925-6)      | . | 59 | Balls (1915a)             | . | 51       |
| Old observation plot  | . | 65 | Prescott (1924)           | . | 56 to 48 |
| New observation plots | . | 68 | Bailey and Trought (1926) | . | 52       |

All values are for Sakel excepting that of Balls which is for Assili.

Whereas in Egypt the maturation period averaged about 52 days, in the Sudan it was from 7 to 16 days longer. Bailey and Trought (1926) stressed the constancy of the period in Egypt and suggested that the variation reflected in Prescott's data (1924a) arose from the inclusion of diseased bolls. On the other hand, Lambert (1925-6) found for the Sudan considerable change during the season, ranging from 70 to 44 days. This contrast between the countries in both the length of the maturation period and the degree of change in length during the season may be ascribed directly to differences in temperature. Balls (1912) quoted Allard as the first to observe that the maturation period of the cotton boll lengthened as the temperature fell. Zaitsev (1927) found a highly significant negative correlation of maturation period and temperature, when comparing flowers from different sowing dates, a lowering of the temperature by  $1^{\circ}\text{C}$ . causing a lengthening of the period by 3 to  $3\frac{1}{2}$  days. In the present comparison, during the 2 months of most rapid boll development the minimum temperatures averaged  $14.8^{\circ}\text{C}$ . for the Sudan winter and  $18.3^{\circ}\text{C}$ . for the Egypt summer, which, on Zaitsev's calculation, would cause a prolongation of about 11 days in the Sudan as compared with Egypt. This corresponds closely with the average of the observed difference noted in Table VI. The wide range of maturation periods in the Sudan can be ascribed largely to the varying temperatures experienced during the bolling season for, as Lambert (1925-6) showed, the earliest and latest bolls develop during periods of higher mean temperatures than the mid-season ones, which ripen during the coldest weather. Although in Egypt the temperatures during the bolling season are less variable, constancy of the period as found by Bailey and Trought would not be expected.

Thus in both countries low temperatures exerted a delaying action on the crop. That this effect operated at the beginning of the season in Egypt and at the end in the Sudan arose directly out of the contrast in the seasons in which the two crops are grown.

Apart from the differences in the length of the period of maturation the boll characters in the two countries were similar. The average weight of seed cotton (seed plus lint) per boll for all healthy bolls is included in Table VII along with similar averages for the weight of 100 seeds after removal of lint,

ginning out-turn (the percentage of lint in seed cotton), and lint index (the weight of lint per 100 seeds).

TABLE VII

*Boll Characters*

|                                    | Sudan.                      |                            | Egypt.     |          |
|------------------------------------|-----------------------------|----------------------------|------------|----------|
|                                    | Old obs. plot<br>(11 years) | New obs. plot<br>(3 years) | Unmanured. | Manured. |
|                                    |                             |                            |            |          |
| Wt. (gm.) of seed cotton per boll. | 2.03                        | 2.63                       | 2.70       | 2.92     |
| Wt. (gm.) of 100 seeds             | 10.10                       | 11.10                      | 10.65      | 11.40    |
| Ginning out-turn                   | 30.50                       | 32.60                      | 32.90      | 32.10    |
| Lint index                         | 4.46                        | 5.32                       | 5.25       | 5.40     |

From Table VII it appears that if the Sudan Gezira is represented only by the 11 years' data of the Old observation plot the individual bolls were smaller than in Egypt. But the 3 years' data from the 3 New observation plots showed higher values comparable with those for Egypt, not only for boll size, but also for seed and lint characters. The early years of the Old observation plot included several seasons with small bolls and light seeds and these have offset recent years, when the bolls have been heavier and comparable in size with those, also obtained in recent years, from the New plots.

Nitrogenous manuring increased the yield of the individual bolls in both countries, as seen in Table VII, from comparable treatments within the same experiments. Because of the wider spacing in the Sudan the crop there should have a heavier boll, for both Prescott (1924) and Crowther (1936) found an increase in boll size with wider spacing in Egypt, and Crowther (1941-2) found the same increase for the Sudan. Since no such difference occurred it is evident that conditions in the Sudan were less favourable during the period of boll development than in Egypt, probably because net assimilation rate was lower then.

The similarity between the yields of the two countries extended not only to weight of seed cotton per boll but also to weights of seed and lint, and the main differences between the countries, apart from the length of the maturation period, were therefore confined to those processes of growth which controlled the number of bolls produced.

## YIELD CAPACITY OF THE PLANTS

The capacity of the plant to produce seed cotton may be measured by the proportion of total dry matter which ultimately is present in the fruits. Table VIII, on a basis of oven dry weight, gives the amount of yield, expressed as seed plus lint, divided by the amount of total dry matter of the entire aerial portions of the plant at the beginning of boll opening. The value thus derived may be termed the 'fruiting coefficient'. Hosking (1937) used the

term 'vegetative efficiency' in a similar sense, but his calculation was based on fresh weights at the *end* of the season.

TABLE VIII

*Fruiting Coefficient*

| Sudan.                              |       | Egypt.           |       |
|-------------------------------------|-------|------------------|-------|
| Old observation plot.<br>(11 years) | 0.437 | 1935 experiments | 0.285 |
| New observation plots<br>(3 years)  | 0.386 | 1936 experiments | 0.386 |

Table VIII gives the fruiting coefficients for cotton, unmanured except in the Old observation plot, and shows that those in the Sudan were as high or higher than those in Egypt, reflecting as great if not greater efficiency in the former country in the utilization of vegetative growth for crop production.

This conclusion is important in a consideration of the causes of yield fluctuations in the Sudan Gezira. In the early years of the irrigation scheme the low yields were attributed to factors operating in mid-season or later. Thus Walley (1924-5), referring to the sharp decline in humidity during October and November, stated: 'The rapid fall in humidity which was greater than usual had a bad effect on the cotton crop.' Abnormally cold winters, November and December, were also blamed (Walley, 1926-7). Later Lambert (1936-7) and Crowther (1941) showed that the crop of the Sudan Gezira was largely predetermined by the rate of vegetative growth during the first weeks after sowing. Table VIII shows that this vegetative growth was utilized efficiently and, since these calculations are based on the data for 11 seasons, it is evident that the factors which limited crop yield in the Gezira were primarily those operating early in the season and governing plant size rather than those affecting the development or shedding of fruits. In the years when the Gezira yields were exceptionally low, i.e. 1930-1 and 1932-3, the fruiting coefficients on the Old observation plot were *above* normal, namely 0.638 and 0.540, indicating that the yield was more than commensurate with the amount of vegetative growth, and that the crop failure was primarily the result of poor vegetative growth.

The conclusion that the Gezira crop is at least as efficient as that of Egypt in the utilization of vegetative growth for crop production is confirmed by the nitrogen analyses. What may be termed the yield coefficient for nitrogen absorption, i.e. the final yield, on an oven-dry basis, divided by the amount of nitrogen present in the aerial portions of the crop at the time of boll-opening, both expressed in kg., is shown for Egypt and the Sudan in Table IX.

This coefficient has also been utilized by Hosking (1937) and termed 'nitrogen efficiency'. Although the crop in the Sudan absorbed less nitrogen during the season than that in Egypt (Table IV) the coefficient was higher

in the Sudan, demonstrating that the yield per unit of nitrogen absorbed was greater there.

In contrast with the Sudan, adverse conditions during later growth in

TABLE IX

*Yield Coefficient for Nitrogen Absorption*

| Sudan.                      |      | Egypt.                 |      |
|-----------------------------|------|------------------------|------|
| Old observation plot . . .  | 25.6 | 1935 experiments . . . | 17.1 |
| (10 years)                  |      |                        |      |
| New observation plots . . . | 25.4 | 1936 experiments . . . | 22.2 |
| (3 years)                   |      |                        |      |

Egypt take on an added importance through the curtailment of the season by bollworm infestation. Whereas, as Figs. 5 (*f*) and 7 (*c*) show, in both countries at the time of the opening of the first bolls there were similar proportions of total dry weight and of nitrogen accumulated in the developing fruits, in Egypt much of the dry weight was present in flower-buds and bolls which failed to survive to normal maturity, so the final proportions were lower than those determined earlier. The result was a lower fruiting coefficient and a lower yield coefficient for nitrogen absorption than in the Sudan.

## CONCLUSIONS

From this detailed comparison of the development of the cotton crop as shown by experiments in the Sudan Gezira and in the Egyptian Delta, it is now possible to indicate the principal factors which control growth throughout the season in the two countries and to compare their operation.

The difference in the appearance of the two crops in the field arises from several causes. The wider spacing of the Sudan allows of taller plants, and the rapid early growth results in longer and thicker internodes, especially at the base of the plant. The tendency towards 'legginess' in the Sudan is enhanced in cotton severely damaged by blackarm by premature defoliation, and occasionally by loss of fruiting branches.

Another difference which has contributed to the impression that the Gezira plant grows under exceptionally unfavourable environmental conditions is the anthocyanin development which is common in the leaves and stems of Sakel during late growth. The effect appears to be the result of high temperatures acting upon leaves in which carbohydrates have accumulated during senescence and where percentage nitrogen is low. The reddening is more general on unmanured than on manured cotton, and in those seasons where maximum temperatures during leaf senescence are unusually high. Such reddening is not unknown in Egypt, for Gracie and Khalil (1935) stated that 'red leaf' was extremely common in 1931 but absent in 1933, the former season being hotter than the latter during the period of senescence. They ascribed the effect to carbohydrate accumulation as a result of water strain.

Because of the high initial rate of growth the Sudan crop passes rapidly

through the purely vegetative stage, and the prolonged picking season renders the duration of leafiness short compared with that of 'legginess'. In Egypt the vegetative phase is prolonged by cold weather and the picking season is curtailed, so that for most of the season the general appearance of the plant is leafy. These differences in appearance are, however, only superficial.

It has been amply demonstrated that the cotton plant is very sensitive to temperature, and, by reason of the difference in the sowing season, the effects of temperature are thrown into relief by a comparison of the two countries. The conclusion of Balls (1912) that germination and early growth in Egypt are controlled by the low night temperatures is borne out by the data presented here. Temperatures in the Sudan at sowing are near the optimum, and germination and early growth proceed at a rapid rate so that, by comparison, a delay of about 30 days in the development of the crop is incurred in Egypt, and this difference in plant growth relative to age persists. The lower temperatures in the Sudan at a later stage prolong the period of boll maturation by about 7 to 14 days, thus partially offsetting the initial delay in Egypt.

While the above are the most pronounced effects of temperature, Gracie and Khalil (1935) stressed the fact that, despite the relative constancy of the Egyptian climate from year to year (Balls, 1938), abnormally hot weather during late growth reduced yield appreciably. Since it is of the highest importance in that country that the bolls should mature before attack by pink bollworm ends the cotton season, and since early growth is delayed by low temperatures, the growing season in Egypt is extremely short, so short that variations in temperature have an importance in no way paralleled in the Sudan. Gracie (1939) points out that the Physical Department in Egypt found that over the 25 years prior to 1939 there had been a rise in the mean annual temperature of the country of  $1^{\circ}\text{C.}$ , and he concluded that this had appreciably raised the average cotton yield.

Although there is some evidence that temperatures during early growth in the Sudan are supra-optimal, and may restrict the rates of both elongation of the main stem and increase in leaf dry weight and also delay the formation of fruiting branches, there is no indication from the data that these high temperatures cause excessive shedding of flower-buds or appreciable reduction in yield.

*Water supply* in both countries is by artificial irrigation at regular intervals, and thus the phenomenon of drought, frequent with rain-grown crops, is absent.

Much work has been done by Greene and Snow (Greene, 1928 and 1936; Greene and Snow, 1935) on the effect of irrigation on Gezira soil. This has demonstrated not only the low permeability to water of Gezira soil, but also the small amount of water available to the crops under existing irrigation practice. In general, increased water supply leads to slightly increased yield. If nitrogen is also added these increases are larger (Gregory, Crowther, and



Lambert, 1932); but when rain falls on newly irrigated cotton land excess water in the upper layers of the soil during early growth may reduce the crop through waterlogging. This, though locally a major factor in some seasons, cannot be considered very important in an average year, and its effects are not discernible in the data presented here.

In Egypt, with its curtailed growing season, Templeton (1932) showed the importance of an additional irrigation given 3 weeks after sowing to encourage early growth, and Balls (1912) stressed the depressing influence of excess water during the period of flowering and bolling caused by the rising of the water-table following the Nile flood.

Despite the pronounced differences in the rate of soil water movement between the two countries and of the association of the lower permeability with the hotter, drier climate, there emerge from the developmental data of effects surprisingly few indications definitely attributable to water supply within the limits prescribed by commercial cultivation.

The importance of *air humidity* has been much stressed in Egypt as elsewhere, and Balls (1912) ascribed the shedding of flower-buds to water strain associated with high temperatures and low humidity, especially when water absorption from the soil was affected by delayed irrigation or by root asphyxiation. Any effect of low humidity found in Egypt should appear in the Sudan and in a more acute form, since during the period of the production of flower-buds humidity is especially low and temperatures especially high. The observations recorded in this paper show *less* shedding of the potential crop in the Sudan, and it is concluded that air humidity has little direct effect on the total amount of shedding in either country. Although there is no evidence of effects of humidity on shedding of flower-buds it is possible that, arising out of the influence of water supply upon extension growth, a high saturation deficit may lead to smaller cells and therefore not only to shorter internodes in the main stem but also to smaller and thicker leaves, with the resulting low rate of assimilation per unit weight of leaf. From the data available it is impossible to state whether temperature changes alone explain adequately these effects or whether humidity also is involved.

Finally, the importance of *nitrogen supply* as a factor limiting growth is established for Egypt as well as for the Sudan. This conclusion on the importance of nitrogen supply to the cotton plant is not confined to the Sudan and Egypt, for Mason (1928) in the West Indies wrote: 'We have in the course of our work been struck by the overwhelming importance of nitrogen in the economy of the cotton plant. The distribution of nitrogen appears to be a most important factor in limiting the growth of the cotton plant.'

Whereas in the Sudan early absorption of nitrogen is rapid, in Egypt low temperatures retard root growth and consequently the rate of absorption of nitrogen. Subsequently the intake of nitrogen is rapid, and it is concluded that in both countries, apart from a short period after sowing, leaf-growth rate is largely controlled by this rate of nitrogen absorption. In both, the evidence supports the interpretation that the number of fruiting meristems

laid down is controlled by the nitrogen supply, whereas the subsequent development of the potential crop is determined by carbohydrate supply.

This conclusion as to the importance of nitrogen supply in Egypt appears incompatible with such statements of Gracie and Khalil (1935) as 'Apart from one or two isolated cases the soil, regarded from the purely chemical point of view of nutrient supply cannot in any way be regarded as a factor limiting the yield of cotton in Egypt'. The writer believes that the difference in view is due to the fact that Gracie and Khalil, explaining why yield response to added nitrogen was the greater on land which normally produced the higher yield, postulated that soil factors which limit yield by affecting the root system of the plant must operate through water strain. The author, on the other hand, would interpret their data in terms of restricted absorption of nitrogen. Balls (1915) recognized the difficulty of disentangling the effects of water and of the nutrient salts dissolved therein. The quotation of Gracie and Khalil given above is taken from a paper describing a series of experiments which gave large increases in yield from nitrogenous manuring, the very existence of which increases shows that nitrogen is one of the factors controlling the yield of cotton in Egypt. In view of the very short growing season now available to the cotton crop in Egypt, the largest increases in yield will result from those factors which hasten growth and boll maturation, such as variety, temperature, spacing, method of sowing, and early irrigation. Yet, although added nitrogen leads to a prolongation of the flowering curve, which means that the additional bolls produced are mainly late ones, the season is sufficiently long to allow of large gains in crop from manuring, as abundantly illustrated in the data both of Gracie and Khalil and of the author. This is in contrast with the experience in South Africa discussed earlier, where *no* response to nitrogenous fertilizer was obtained.

The environment in the Sudan leads to rapid germination and early growth, and with a soil deficient in nitrogen the rate of nitrogen absorption early reflects any difference between seasons in the amount available to the crop. This gives rise to the correlation of early leaf nitrogen and final yield. The existence of this correlation in cotton which is manured each year with ammonium sulphate and where the only other crop in the rotation is a legume emphasizes the extent to which growth in the Gezira is controlled by the nitrogen factor.

Factors which affect nitrogen supply in the soil will affect growth indirectly, for example, temperature, humidity, and other influences of the weather. The violent fluctuations in the Gezira yields during the 22 years up to 1933-4 have been shown to be correlated with differences in the amount and distribution of rainfall (Crowther, E. M., and Crowther, F., 1935), and it is apparent that the crop in the Gezira is controlled by soil factors to a degree greater than that in Egypt. That the reserves of nitrogen in the soil available for crop production are more easily maintained in Egypt is clear, since the land is rarely left uncropped for more than a few months at a time; whereas commercially in the Sudan Gezira it has been found necessary to leave the

land uncropped for a long spell before cotton is sown. The period at present is 20 to 27 months, yet despite the resultant accumulation of nitrogen the crop shows a marked sensitivity to nitrogen supply, exemplified in its response both to conditions of the land during this uncropped period (Crowther, 1943) and to nitrogen added to the soil by manuring of the cotton (Crowther, 1941a).

#### SUMMARY

This account of observations on morphological characters, on production of dry matter, on absorption of nitrogen, and on yield, all recorded from replicated experiments, furnishes a comparison of the development of the cotton crop in the Sudan Gezira and in the Nile Delta. The two areas, both irrigated from the Nile, are 1,200 miles apart, and the contrast in their climates is accentuated by the difference in season, the Sudan crop being a winter one and the Egypt a summer one.

There were marked differences in early growth which was rapid in the Sudan, but in Egypt retarded by low temperature. The growing season in Egypt, initially limited by low temperature, was also prematurely curtailed by bollworm infestation which destroyed the late crop while temperatures were still favourable. This, in Egypt, placed a premium on earliness which was absent in the Sudan, where climatic conditions for early growth were near the optimum and where no external factor interfered drastically with late growth.

In Egypt an early maturing crop is achieved in part by the adoption of a spacing much closer than formerly and closer than that practised in the Sudan Gezira. It was mainly this closer spacing which caused the crop in Egypt to be borne on the lower nodes of the main stem. In the Sudan the crop was borne higher up the main stem, and, though the primary cause of the higher fruiting was the wider spacing, both high temperatures at the time of the formation of fruiting branches and blackarm where severe contributed to this effect.

Leaf growth was closely associated with rate of absorption of nitrogen, the relative leaf-growth rate in both countries being highly correlated with percentage of nitrogen in the leaf dry weight. Nitrogen absorption relative to plant size was more rapid during early growth in the Sudan than at any time in Egypt. At this period in the Sudan any differences between seasons in amount of nitrogen supply were soon reflected in the nitrogen composition of the plants; thus there arose the significant correlation, found previously, between the percentage nitrogen in leaf dry matter shortly after sowing and the final yield.

Explanation of the flowering curves in terms of nitrogen, previously advanced in interpretation of the Sudan data, applied equally well to Egypt; and there was no evidence that the Sudan plant, because of extreme environmental or soil conditions, suffered undue fruit shedding as compared with Egypt.

Maturation of the bolls was delayed in the Sudan by low winter temperatures. This delay in the opening of the bolls, together with that which arose from the wider spacing, almost equalled the initial delay in Egypt occasioned by the low spring temperatures there.

The yield capacity of the Sudan Gezira crop, judged in terms of production per unit of either vegetative growth or nitrogen absorbed, was as efficient as or even more efficient than that of the crop in Egypt. Thus there was no evidence for the view that yield in the Gezira was principally limited by any factor operating during late growth. Yield in the Sudan appeared to depend more upon soil factors than did yield in Egypt and to be closely related to nitrogen supply. Early growth in the Sudan was rapid and the supply of nitrogen from the soil soon ceased to be adequate to maintain the high initial rate of uptake, and thus nitrogen became limiting. This limitation arose despite the opportunity for accumulation of nitrogen afforded by the long resting period of 20 to 27 months which preceded the cotton sowing, in contrast with the few months allowed in the rotation in Egypt.

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# A Consideration of the Factors controlling the Opening of Buds in the Cacao Tree (*Theobroma Cacao*)

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With three Figures in the Text

## I. INTRODUCTION

PERIODIC renewal of bud activity is a characteristic phenomenon in many tropical trees, resulting in the rapid production of a new set of leaves. This commonly occurs in the cacao tree in Trinidad and elsewhere, and a new 'flush' of leaves is said to be produced. The new leaves attain their full size in the course of about four weeks after bud opening.

A knowledge of the factors causing flushing in the cacao tree is desirable from several points of view. Certain insects, especially cacao thrips (*Selenothrips rubrocinctus*), exhibit a preference for the young leaves, while the young developing buds are often the seat of infection for witches' broom disease (Baker and Crowdy, 1943). It is also known that flushing may divert nutrient materials from the developing fruits, and in young trees especially it may thus cause considerable fruit wilt (Humphries, 1943*a*).

Hitherto investigations on the periodicity of flushing have failed to find any consistent connexion between the time of flushing and meteorological or other conditions. MacDonald (1932, 1933) suggested that flushes coincide with the onset of drier conditions following a wet spell or coincide with a wet spell following dry weather, i.e. a rise in soil moisture, but it is obvious that this explanation does not meet all cases. Pound (1933) was able to associate only one flush out of four with weather changes; and later he showed (1936) that applications of ammonium sulphate, sulphate of potash, and superphosphate alone or in combination had no effect on the intensity or periodicity of flush.

## II. METHODS AND PROCEDURE

In order to record the frequency of occurrence of bud opening in the cacao tree two methods were adopted. The first, which might be called the intensive method, was to mark a number of dormant buds on each of the experimental trees and to record their vegetative condition at 14-day intervals. For this purpose three trees at River Estate (subsequently referred to as estate A), constituting clonal-budded and grafted material already the subject of various experiments (Humphries, 1943, 1943*a*), were selected. During the



first year of the experiment 20 buds were kept under observation on each tree but subsequently the number was increased to 30, to provide a more representative sample. There was a slight loss of buds from time to time, especially towards the end of the dry season.

The second method enabled a larger number of trees to be kept under observation. Here the trees were individually inspected every week and given marks ranging from  $\frac{1}{2}$  to 6 according to the intensity of flush. The scale of marks (cf. Pyke, 1933) ranged as follows:

0. No flush.
- $\frac{1}{2}$ . Less than 10 flushes on the whole tree.
1. Some, but comparatively, few flushes.
2. Flushes over about one-third of the tree.
3. Flushes over about one-half of the tree.
4. Flushes over about two-thirds of the tree.
5. Flushes over nearly the whole tree.
6. Flushes over the whole tree.

A plot of 14 trees at estate A were assessed by this method. This plot consisted of clonal material of which the three trees mentioned above as having been assessed by the intensive method form a part. A number of trees at La Reconnaissance Estate, Lopinot (estate B) were also observed by this method; there were 3 plots of mature estate trees (not clonal) with 15 in one plot and 8 in each of the others. In order to assess the average behaviour of the trees in each plot from week to week, the mean of the marks was taken. This constitutes therefore a general method in contrast to the intensive method already described, and the two methods provide a convenient check against one another.

### III. RESULTS

#### (a) *Intensive method.*

The data obtained by this method from trees at estate A have been plotted in Fig. 1 (first year) and Fig. 2 (second year). The number of buds opening at any given time has been expressed as a percentage of the total number of healthy (marked) buds still present on the tree. This percentage has been taken as a measure of the flushing intensity and is indicated by upright columns in Figs. 1 and 2. It will be noticed that a big flush occurred on the three trees soon after the commencement of observations (May–June 1940), and that subsequently there was a less intense flush in September–October. There was no further bud activity on any of the trees until February of the next year, when almost all the marked buds opened at the same time. After an interval of about 7 weeks a lighter flush occurred which was followed after a similar interval by yet another flush (see Fig. 2, June 1941). The next flush occurred in September–October with a very slight flush on two of the trees in November. In the following January there was another flush on all the trees,

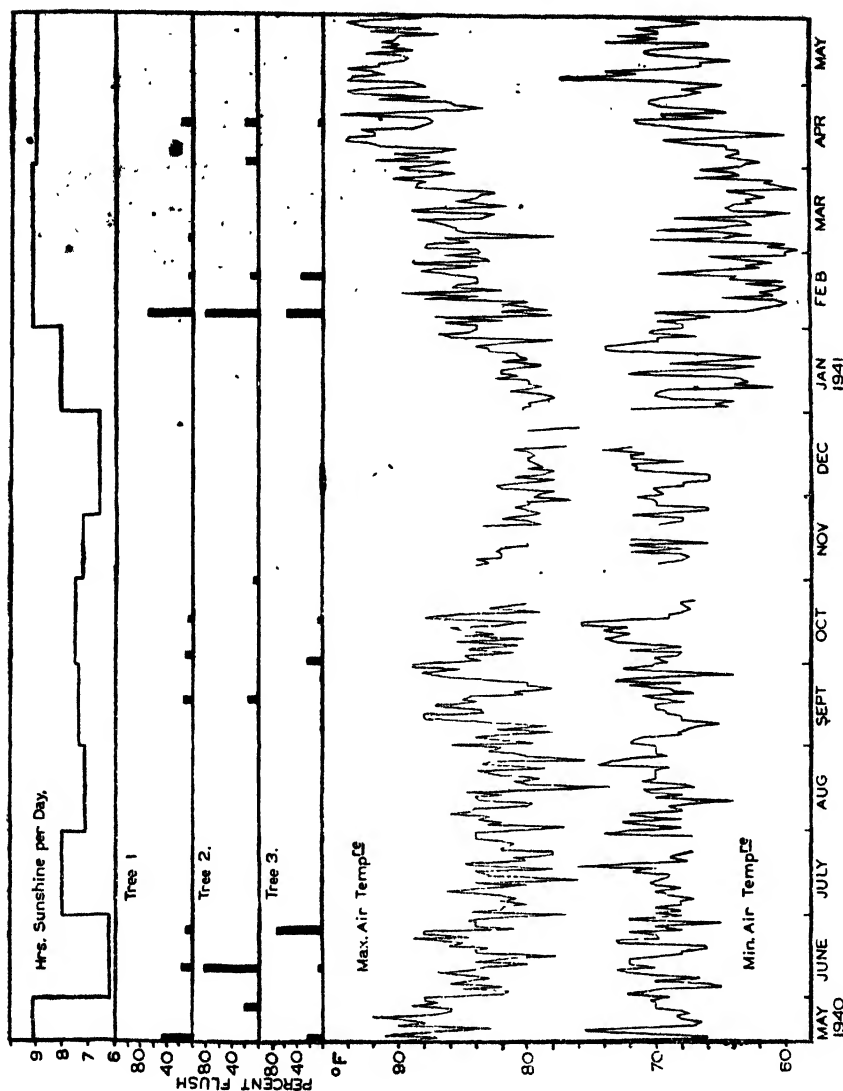


FIG. 1. The daily maximum and minimum shade temperatures, hours of sunshine per day (monthly average), and percentage of buds opening on three trees at estate A during the first year of observation.

somewhat earlier than in the previous year. Subsequently two more flushes followed at about 7- or 8-week intervals as in the preceding dry season.

From the above it is not possible to discover how frequently the terminal bud on any particular twig is capable of producing a new flush. This may, however, be obtained by an analysis of the data of individual buds. It was found that some branches flushed at quite frequent intervals, for example, one terminal bud burst on March 17 and again on April 14, an interval of only 4 weeks. This was the shortest period recorded during the experiment, but frequent flushing at fairly short intervals was by no means uncommon at the height of the dry season, the interval between flushes ranging from 6 to 8 weeks, and this appears to be the minimum period.

In order to discover if periods of flush are in any way connected with environmental conditions, certain meteorological observations were recorded at the experimental sites. A thermo-hygrograph (Negretti and Zambra type) was maintained in a Stevenson screen adjacent to the trees under observation, and the temperatures recorded therein were checked at weekly intervals against a standard thermometer. The soil temperature at 3 in. depth was also recorded. Soil samples were collected weekly for moisture determinations and rainfall figures were abstracted from the estate records. Since it is impossible to present all the meteorological data, a selection has been made in Figs. 1 and 2 where the flushing frequencies are also shown. The daily maximum and minimum temperatures are taken from the continuous temperatures records. The monthly average hours of sunshine per day were abstracted from records at the Imperial College of Tropical Agriculture, being the only sunshine records available in Trinidad; in all probability they are quite representative of conditions on the estate. There is no obvious relationship between the duration of sunlight and the frequency of flushing, since this often occurs in the middle of the dry season (February and March) when the average duration of sunlight is as high as 9 hours per day, or in September and October when it may be as low as 6 hours. It was also concluded that flushing frequency bore no relationship to soil moisture conditions. Flushing occurs most frequently at the height of the dry season when the soil moisture content is very low, but may also occur during the rainy season when soil moisture is high. The question arises as to whether the length of day has any connexion with flushing frequency. The variation in the day length in Trinidad is not very great, the maximum being about  $12\frac{3}{4}$  hours and the minimum about  $11\frac{1}{4}$  hours. The duration of daylight is 12 hours or over between March and September and at a minimum in December. It will be seen from Figs. 1 and 2 that flushing occurs both during the short days (Jan.-Feb.) as well as during the long days (June).

There appears, however, to be a close relationship between *flushing frequency and maximum temperature*. At the time that observations were begun (May 1940, Fig. 1) the daily maximum temperatures were high, but fell gradually until the beginning of July, when they remained fairly steady until the end of August. During this period of high temperatures most of

the buds opened. In September and October the maximum temperatures again rose and another flush occurred. In November–December temperatures were again lower and no flushing occurred. In January (1941) with the onset

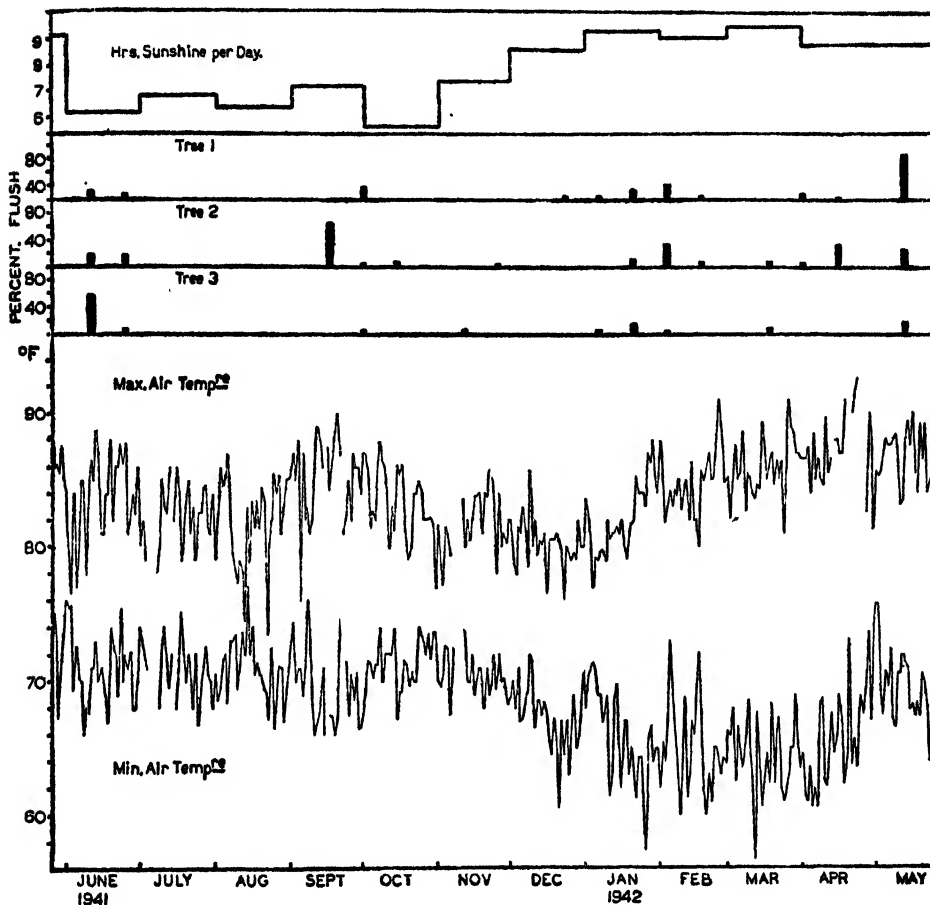


FIG. 2. The daily maximum and minimum shade temperatures, hours of sunshine per day (monthly average), and percentage of buds opening on three trees at estate A during the second year of observation.

of the dry season the maximum temperatures began to rise again, reaching a peak in April and May and falling off again in June. During this long period of high temperatures there were three distinct periods of bud activity. After the dry season there was no further activity until September–October (Fig. 2), when a further spell of high temperatures occurred and there was then a slight flush on all three trees in the middle of this period. Apart from two very slight flushes in November, affecting only a few branches on two of the trees when there was a slight rise in maximum temperatures, the next flush occurred in January 1942, earlier than in the previous year. It is interesting

to note that the dry season of that year began about the middle of the previous December, which is abnormally early.

It is apparent from the data that flushing is very closely connected with periods of high maximum temperatures. When the maximum temperature is above about  $83^{\circ}$  F. (in the screen), conditions appear to be favourable for renewed bud activity. Minimum temperatures do not appear to show such a close relationship with flushing periodicity.

*(b) General method.*

The results obtained by this method are shown in Fig. 3, where the average weekly marks for the flush of all the trees and the weekly average maximum shade temperatures have been plotted. As already mentioned, the 14 clonal trees were kept under observation at estate A, while 3 groups of ordinary estate cacao trees were used at estate B. Essentially the same results were obtained in all cases, except that the mark system gave indications of a slight flush at the periods of lowest maximums (Nov.–Dec.) in both years. Inspection of the assessments of the individual trees showed that the fairly high marks for these two periods were largely due to the behaviour of three particular trees. It is possible that defoliation due to thrips attack caused flushing during these periods, but unfortunately no records of insect incidence were kept. No flushes at these particular times were recorded on the trees under intensive observation.

The data for trees on estate B (Fig. 3) represent the average for a plot of 15 trees. The results for the other two plots are essentially the same and are not here represented. Again there is a striking connexion between the maximum air temperature and the degree of flush on the tree. When the maximum temperatures are high (and especially at the commencement of a period of high maximum temperatures) there is a considerable amount of bud activity. When temperatures are minimum, however, there is practically no flushing. It should be noted that, since the lowest mark given for flushing intensity was  $\frac{1}{2}$ , lines have been drawn at this level in Fig. 3; indications of flush below this level may be neglected.

The evidence presented definitely indicates that maximum temperature is closely connected with bud opening in cacao. When the maximum air temperature (as indicated by thermometers in a Stevenson screen under the trees) is above a certain level (which in the cases recorded appears to be about  $83^{\circ}$  F.), conditions are apparently suitable for renewed bud activity. When the maximum temperature is below this, normal flushing rarely, if ever, occurs. All the temperatures recorded were those of the air in the screen; no records were made of the temperature at the leaf or bud surface, which will presumably be somewhat higher in the buds, if not in the leaves, especially in the dry season when the shade trees (*Erythrina*) are leafless, although this would lead to increased exposure of the screen also. In a well-shaded plot, however, it is reasonable to suppose that the leaves have *approximately* the temperature of the air.

#### IV. DISCUSSION

Owing to its great importance, especially in fruit trees, a good deal of attention has been paid to the subject of bud dormancy. It has been found that diverse agents may cause dormant buds to renew their activity. These

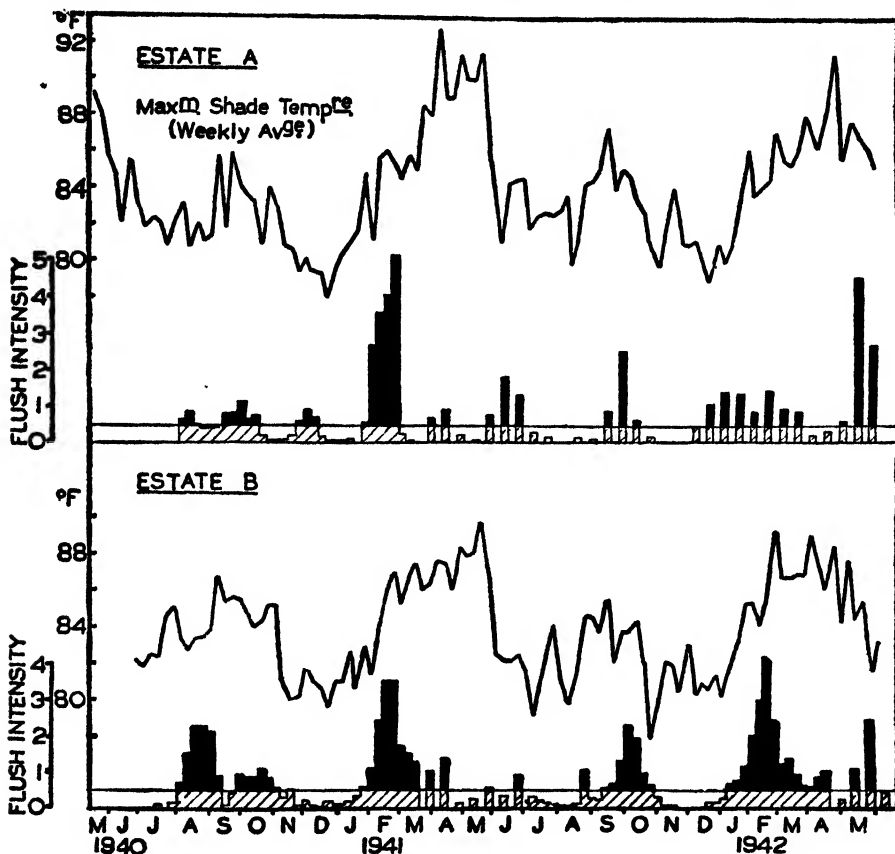


FIG. 3. The weekly average maximum shade temperatures and average degree of flush on experimental trees at estates A and B during a 2-year period.

include warmth, cold, X-rays, acids, certain oils, ether, alcohol, chloroform, ethylene, thiourea, sodium thiocyanate, sodium nitrate, injection of water, diastase, and other chemical substances, while wounding or mere mechanical stimulation have been sufficient in some instances. Recently Guthrie (1940) reported that ethylene chlorhydrin treatment increases the glutathione content and shortens the rest period of dormant potato tubers. Direct treatment with glutathione is equally effective. Similar results were obtained with resting pear and peach buds. Whether these agents act on a particular metabolic system or on different systems producing the same ultimate effect (namely, stimulation of dormant buds) it is not possible to decide on the

evidence available. Some of the earliest work on dormancy (Molisch and others) showed that immersion of dormant twigs in warm water for a short period (9–12 hours) caused dormant buds to burst and grow, provided subsequent conditions were favourable for growth. Molisch found that a temperature of 30° C. (86° F.) was the optimum temperature for a number of species, but 35°–40° C. was necessary for others. Furthermore he found that the response was confined to buds actually submerged in the bath; i.e. the effect was very localized. It was further shown that the effect could remain latent until a time when conditions again became favourable for growth. It is possible that bud activity in cacao is governed by a similar process. Many analytical studies have been made of the chemical changes taking place during dormancy and subsequent bud opening, but they have not fully explained the mechanism by which various treatments bring about termination of the rest period. They are all responsible apparently for inducing a certain internal condition necessary for the initiation of growth, and the observations of Guthrie (1940) that glutathione is produced in resting buds by ethylene and thiourea treatment is a big advance in explaining the phenomenon. Guthrie's observations also fall into line with those of Hopkins and Morgan (1943), who show that the appearance of sulphhydryl compounds during seed germination is apparently universal among plants, and moreover that these compounds seem to prepare the conditions for the growth of the embryo rather than to be produced under its influence.

Much less is known about the growth responses of tropical trees than of temperate trees, and it may well be that periods of high temperature are as essential for renewal of bud activity in some tropical trees as periods of low temperature are for certain temperate trees. Casual observation indicates that other tropical trees such as the mango (*Mangifera indica*) behave similarly in their flushing habits. In temperate trees the minimum dormant period necessary before renewal of activity appears to be quite long, but in cacao the minimum dormant period is very short, in fact, probably as long as is necessary for the new terminal bud to reach maturity and at the same time to mobilize sufficient food reserves, a period of about 7 weeks or less. This must be due to the fact that metabolic changes are much more rapid at tropical temperatures and there is no period of the year which is unfavourable to growth. Even in the dry season, when soil moisture is at a minimum, vigorous flushing may take place.

It is possible that high temperature *per se* is not the factor responsible for inducing bud activity, since high temperature is correlated with other factors such as light intensity, internal pressure deficit, &c., one of which may be the real causal agents of flushing. It is conceivable, however, that a mere rise of temperature could bring about changes in the plant cell, such as increase in glutathione content, which would initiate growth.

The view here put forward, that flushing in cacao is an effect of high temperature, is supported by certain general observations. Cacao trees on the outside of a plantation tend to flush more frequently than those inside,

possibly because of more direct insolation. Flushing also occurs more frequently in 'pockets' caused by the falling of cacao and shade trees, and is more marked under inadequate shade in the dry season. It seems clear that measures which tend to reduce air temperature will be instrumental in reducing flushing intensity. The above theory suggests why it is possible to have flushing both in the dry and in the wet seasons although conditions of rainfall, soil moisture, and atmospheric humidity in these two seasons are directly opposed. This was previously the chief obstacle to any rational explanation of flushing frequency.

## V. SUMMARY

1. Factors affecting the breaking of the rest period (flushing) of buds of *Theobroma cacao* have been investigated over a 2-year period.
2. A close relationship was found between flushing frequency and maximum shade temperatures.
3. No obvious relationship was found to exist between flushing frequency and minimum temperatures, length of day, hours of sunshine per day, soil moisture, soil temperature, or rainfall.
4. The minimum resting period for a cacao bud was found to be about 7 weeks.

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# The Cytology of *Salix* in Relation to its Taxonomy

BY

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With twenty-six Figures in the Text

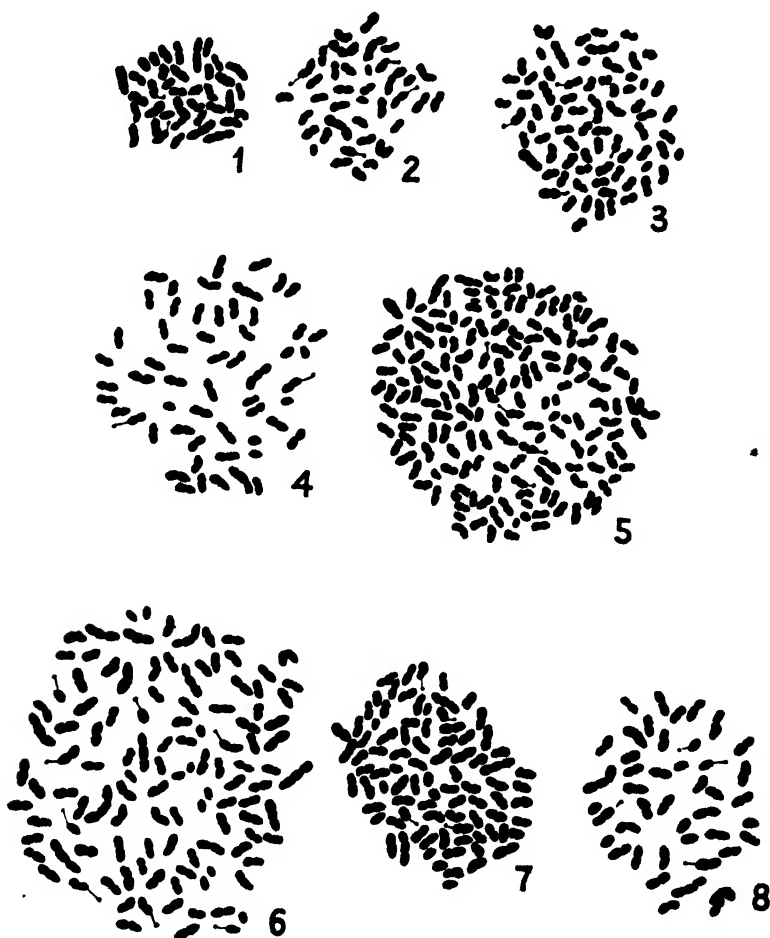
## INTRODUCTION

IN a detailed study of the cytology of a group of *Salix* species and hybrids (Wilkinson, 1941) the writer showed that certain chromosomes present in the somatic metaphase complement could be used as taxonomic indicators, and that  $F_1$  hybrids between well-established species could be detected by an application of the effect known as 'amphiplasty' (Navashin, 1934). It seemed to the writer that an application of these methods to a wider range of *Salices* might yield results of value in elucidating their taxonomic and even phylogenetic interrelationships, and the results of such an extended study form the basis of this account. Such an attempt to clarify systematic relationships in this intricate genus is abundantly justified in view of the notorious diversity of conclusions expressed by various workers pursuing other lines of evidence. Thus, for example, Fisher (1928), from a consideration of floral morphology and anatomy, concluded that the synandrous, diandrous, and pleiandrous willows respectively form an increasingly primitive series approaching the poplars. This contrasted with views previously advanced by Hold  n (1912) who, in a critical study of wood anatomy, concluded that primitiveness was manifest rather in the opposite direction.

Some sixteen species of *Salix*, mostly British, have as yet been reported upon cytologically. The most comprehensive contribution to date is that of Blackburn and Harrison (1924), which forms a sound basis for the further consideration of phylogenetic relationships in the genus. In most of the outstanding examples of the application of karyology to taxonomic study, amongst which the work of Babcock and his co-workers on the genus *Crepis* is fundamental, the chromosomes are comparatively large and admit of clear resolution into morphological categories. The *Salices* do not at first attract study from this point of view, as the somatic complements are numerically large and the chromosomes are small and apparently devoid of particularly distinctive morphological features. Nevertheless, detailed analysis of the chromosome complements of 26 species and 10 hybrids has, in the view of the writer, given results of value. Further, intimate study of  $F_1$  and one series of  $F_2$  hybrids has yielded information of considerable interest, especially in relation to the phenomena associated with 'amphiplasty'.

# TECHNIQUE

Several of the world species and hybrids have been obtained from the botanical gardens at Kew, Edinburgh, Lund, and Oslo. The writer has collected in Durham and the Tyne Valley, in the vicinity of Liverpool, in



FIGS. 1-8. Somatic metaphase in *Salix* species. ( $\times 3,000$ .) Fig. 1, *S. hypoleuca*. Fig. 2, *S. gracilistyla*. Fig. 3, *S. lapponum*. Fig. 4, *S. daphnoides*. Fig. 5, *S. glauca*. Fig. 6, *S. Andersoniana*. Fig. 7, *S. phylicifolia*. Fig. 8, *S. livida*.

Gower, and in California. He is also indebted to numerous friends for their kindness in supplying cuttings at various times.

Cuttings from vigorous shoots were used for this study. There is considerable variation from species to species in the time taken for roots to emerge from cuttings placed in water, the pleiandrous willows and those near to them (on Andersson's widely accepted systematic scheme) tending to root

with difficulty. A dilute standard culture solution (Klebs), commonly used for algae, was found to be a suitable medium in which to induce rooting within a reasonable period. Some species, e.g. the northerly *Salix polaris*, *S. arctogena*, and *S. arbuscula*, were never rooted successfully. Others such as *S. Caprea*, *S. pentandra*, and *S. repens* rooted only after long delay.

Experience in the study of the cricket-bat willow group determined the use of Belling's modification of Navashin's fluid as the most appropriate fixative, especially for the examination of constriction regions in the somatic chromosomes. The paraffin-wax technique was adopted, using thin sections ( $6\ \mu$ ) for study of the somatic metaphase chromosomes and thick ones ( $20\ \mu$ ) for the examination of nucleolar behaviour. Iron-alum-haematoxylin, used as previously described (Wilkinson, 1941), proved the most satisfactory general stain. The Feulgen light-green staining technique (Semmens and Bhaduri, 1939) was also of value in nucleolar studies. Drawings of the somatic metaphase plates were made at a magnification of 3,000 diameters, and of the preparations for the study of nucleolar behaviour at 1,800 diameters.

### THE SOMATIC METAPHASE CHROMOSOMES

Tables I and II give the number and morphology of the somatic metaphase chromosomes found in this study in the species and hybrids respectively,

TABLE I  
*Summary of Somatic Chromosome Number and Morphology in Various Salix Species*

| Section and Group. | Species.                  | Chromosome Number. | Base No., &c.   | Metaphase chromosome morphology.            |        |
|--------------------|---------------------------|--------------------|-----------------|---|--------|
| SYNDANDRAE         |                           |                    |                 |   |        |
| Purpureae          | <i>purpurea</i> L.        | *38                | 19 (diploid)    | $2L' + 32M + 2T_m + 2S_o$                   | —      |
|                    | <i>Bockii</i> Seemen      | 38                 | 19 { " }        | "   | Fig. 1 |
|                    | <i>hypoleuca</i> Seemen   | 38                 | 19 { " }        | "   | —      |
| DIANDRAE           |                           |                    |                 |   |        |
| Viminalis          | <i>viminalis</i> L.       | *38                | 19 { " }        | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
|                    | <i>gracilistyla</i> Miq.  | *38                | 19 { " }        | "   | Fig. 2 |
|                    | <i>lapponum</i> L.        | 38                 | 19 { " }        | "   | —      |
|                    | "                         | 76                 | 38 (tetraploid) | $4L' + 60M + 2T_m + 2T_s + 8S_o$            | Fig. 3 |
| Pruinosae          | <i>daphnoides</i> Vill.   | 57                 | 19 (triploid)   | $3L' + 46M + 2T_m + 1S + 5S_o$              | Fig. 4 |
| Nitidulae          | <i>Myrsinites</i> L.      | *38                | 19 (diploid)    | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
|                    | "                         | 190                | 19 (decaploid)  | Not determined.                             | —      |
|                    | <i>herbacea</i> L.        | 38                 | 19 (diploid)    | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
| Niveae             | <i>lanata</i> L.          | *38                | 19 { " }        | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
|                    | <i>glauca</i> L.          | 176                | 22 (octoploid)  | Not determined.                             | Fig. 5 |
| Phylicifoliae      | <i>Anderssoniana</i> Sm.  | *114               | 19 (hexaploid)  | $8L' + 88M + 4T_m + 2T_s + 2T_{so} + 10S_o$ | Fig. 6 |
|                    | <i>phylicifolia</i> L.    | *88                | 22 (tetraploid) | $4L' + 70M + 2T_m + 2T_{so} + 10S_o$        | Fig. 7 |
| Argentae           | <i>repens</i> L.          | *38                | 19 (diploid)    | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
| Hastatae           | <i>cordata</i> Muhl.      | 44                 | 22 { " }        | $2L' + 32M + 2T_m + 2T_{so} + 6S_o$         | —      |
| Capreae            | <i>Caprea</i> L.          | *38                | 19 { " }        | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
|                    | "                         | 76                 | 19 (tetraploid) | $4L' + 60M + 2T_m + 2T_s + 8S_o$            | —      |
|                    | <i>cinerea</i> L.         | *76                | 19 { " }        | "   | —      |
|                    | <i>atrocinerea</i> Brot.  | *76                | 19 { " }        | "   | —      |
|                    | <i>Medemii</i> Boiss.     | 76                 | 19 { " }        | "   | —      |
|                    | <i>aurita</i> L.          | *38                | 19 (diploid)    | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
|                    | "                         | *76                | 19 (tetraploid) | $4L' + 60M + 2T_m + 2T_s + 8S_o$            | —      |
|                    | <i>livida</i> Wahl.       | 44                 | 22 (diploid)    | $2L' + 32M + 2T_m + 2T_{so} + 6S_o$         | Fig. 8 |
|                    | <i>Sieboldiana</i> Bijdr. | 76                 | 19 (tetraploid) | $4L' + 60M + 2T_m + 2T_s + 8S_o$            | —      |
|                    | <i>magnifica</i> Hemsl.   | 38                 | 19 (diploid)    | $4L' + 28M + 2T_m + 2T_{so} + 2S_o$         | —      |
| PLEIANDRAE         |                           |                    |                 |   |        |
| Amygdalineae       | <i>triandra</i> L.        | *38                | 19 { " }        | $2L' + 32M + 2T_m + 2T_{so} + 6S_o$         | —      |
|                    | "                         | 44                 | 22 { " }        | $74M + 2T_m + 2T_{so} + 10S_o$              | —      |
|                    | "                         | 88                 | 22 (tetraploid) | "   | —      |
| Pentandrae         | <i>pentandra</i> L.       | *76                | 19 { " }        | $4L' + 60M + 2T_m + 2T_s + 8S_o$            | —      |
|                    | <i>lucida</i> Muhl.       | *76                | 19 { " }        | "   | —      |
|                    | <i>lasianдра</i> Benth.   | 76                 | 19 { " }        | "   | —      |

\* Denotes verification of number reported by previous authors, principally Blackburn and Harrison.

TABLE II

*Summary of Chromosome Number and Morphology in Selected Hybrids*

| Hybrid.   | Somatic chromosome number. | Chromosome morphology.                |         |
|---|----------------------------|---------------------------------------|---------|
| <i>lappomum</i> × <i>viminialis</i>               | 38 (19+19)                 | $4L' + 29M + 1T_m + 1T_{s0} + 3S_0$   | Fig. 9  |
| <i>herbacea</i> × <i>lappomum</i>                 | 38 (19+19)                 | "                                     | Fig. 10 |
| <i>lappomum</i> × <i>Caprea</i>                   | 38 (19+19)                 | "                                     | —       |
| <i>lappomum</i> × <i>aurita</i>                   | 38 (19+19)                 | "                                     | —       |
| <i>Caprea</i> × <i>viminialis</i>                 | 41 (19+22)                 | $3L' + 31M + 1T_m + 1T_{s0} + 5S_0$   | Fig. 11 |
| <i>aurita</i> × <i>atrocinerea</i>                | 76 (38+38)                 | $4L' + 61M + 1T_m + 1T_s + 1S + 8S_0$ | Fig. 12 |
| <i>Caprea</i> × <i>atrocinerea</i> F <sub>1</sub> | 76 (38+38)                 | $4L' + 61M + 1T_m + 1T_s + 1S + 8S_0$ | —       |
| <i>Caprea</i> × <i>atrocinerea</i> F <sub>2</sub> | 76 (38+38)                 | $4L' + 60M + 2T_m + 2T_s + 8S_0$      | —       |
| <i>purpurea</i> × <i>viminialis</i>               | 38 (19+19)                 | $3L' + 31M + 1T_m + 1T_{s0} + 2S_0$   | Fig. 13 |
|   | 57 (38+19)                 | $3L' + 47M + 1T_m + 1T_s + 5S_0$      | Fig. 14 |
| <i>purpurea</i> × <i>aurita</i>                   | 38 (19+19)                 | $3L' + 31M + 1T_m + 1T_{s0} + 2S_0$   | —       |
| <i>aurita</i> × <i>phylicifolia</i>               | 63 (44+19)                 | $4L' + 50M + 1T_m + 1T_{s0} + 7S_0$   | Fig. 15 |

together with other relevant data. The groups *Fragiles* and *Albae* are omitted, as they have been dealt with elsewhere (Wilkinson, 1941). The following symbols are used for the various types of chromosomes in the complements:

- $T_m$  Constricted, satellited (trabanted) chromosomes in medium length-class.
- $T_s$  Constricted, satellited chromosomes in short length-class.
- $T_{s0}$  Short, satellited chromosome without apparent constriction.
- $L'$  Long chromosome with secondary constriction.
- $M$  Medium chromosome with median constriction.
- $S$  Short chromosome with median constriction.
- $S_0$  Short chromosome without apparent constriction.

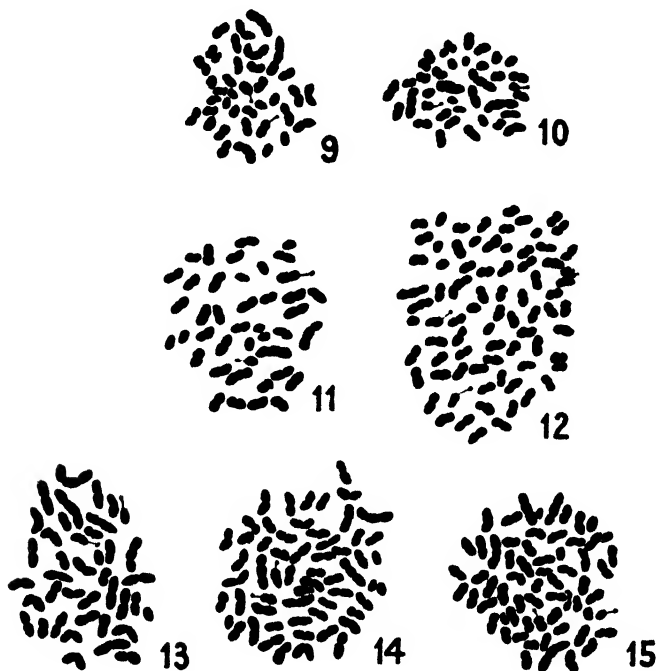
The chief implications of the tabulated results are discussed later.

## THE NUCLEOLAR CYCLE

During the course of the mitotic cycle the nucleolus behaves in a characteristic manner. The nucleolar cycle is well illustrated in a typical diploid species, *S. Caprea*. At the commencement of prophase the large spherical nucleolus is single. It becomes progressively distorted and lobed (Fig. 16), then at a very late stage before metaphase it cleaves into four smaller nucleoli, each with a single chromosome attached to its surface (Fig. 17). These nucleolar bodies disintegrate completely during early metaphase. At telophase they reappear, then merge into one during the reorganization of the daughter nucleus (Fig. 18). The number of attached chromosomes corresponds with the number of satellited chromosomes. The connecting structures between the satellited chromosomes and the nucleolar membranes are too small to permit of any study of their detail.

Throughout the range of species and hybrids studied, the number of nucleoli corresponds with the number of satellited chromosomes. Thus,

both diploid (Fig. 17) and tetraploid (Fig. 19) races of *S. Caprea*, each showing two pairs of satellited chromosomes on somatic metaphase plates, exhibit four nucleoli at late prophase and early telophase; *S. Bockii* (Fig. 20) and *S. purpurea*, synandrous diploids with a single satellited pair, have two nucleoli;

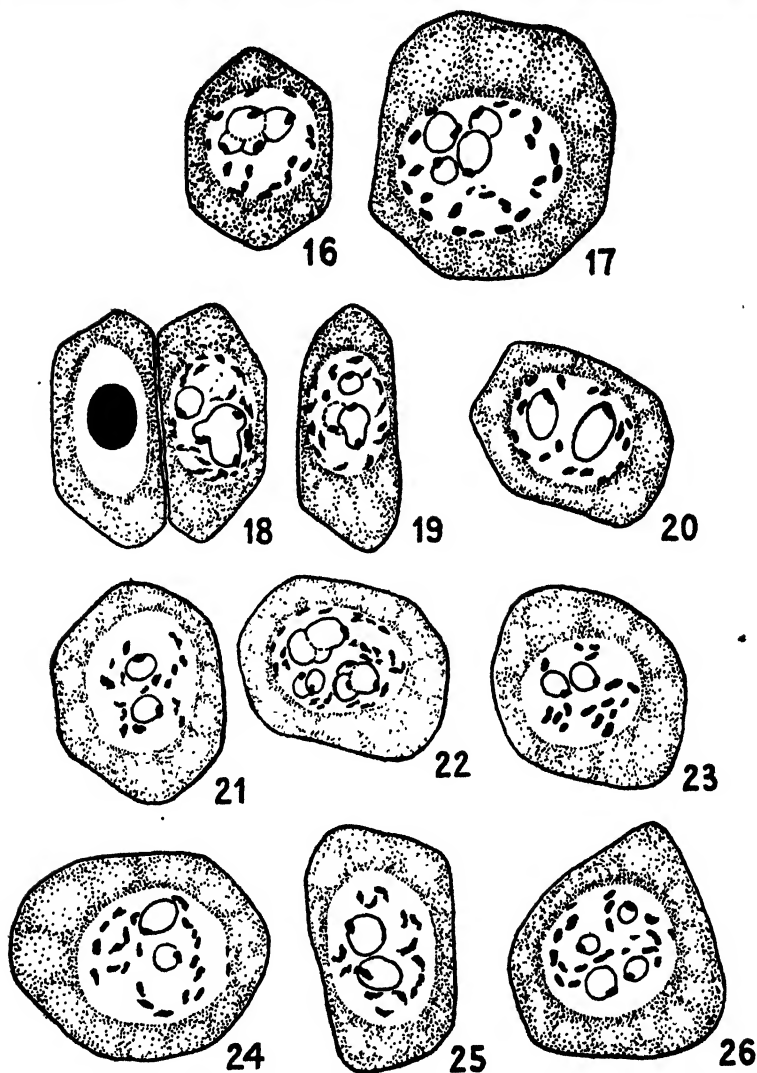


FIGS. 9-15. Somatic metaphase in *Salix* hybrids. ( $\times 3,000$ .) Fig. 9, *lapponum*  $\times$  *viminalis*. Fig. 10, *herbacea*  $\times$  *lapponum*. Fig. 11, *Caprea*  $\times$  *viminalis*. Fig. 12, *aurita*  $\times$  *atrocinerea*. Fig. 13, *purpurea*  $\times$  *viminalis* (= *rubra*). Fig. 14, *rubra* (triploid). Fig. 15, *aurita*  $\times$  *phylicifolia*.

and *S. Andersoniana*, a hexaploid with eight satellited chromosomes, exhibits a maximum of eight nucleoli in the mitotic cycle (Fig. 22).

The loss of satellites in the  $F_1$  hybrids due to amphiplasty is also correlated with nucleolar number. Thus, the diploid *lapponum-aurita* (Fig. 23) and the tetraploid *Caprea-atrocinerea* (Fig. 25) show two nucleoli; whilst the triploid strain of *S. rubra* (Fig. 24) also shows two nucleoli, suggesting that the satellite of the *purpurea* parent has been lost in transmission to the progeny. On the other hand, the tetraploid  $F_2$  *Caprea-atrocinerea* (Fig. 26) shows four nucleoli at late prophase, as in either of the original parents, indicating that this feature of the amphiplastic effect is not transmitted to the second hybrid generation.

It may be noted in passing that the supposedly pure *daphnoides*, here recorded as a triploid, shows in its satellite and nucleolar number (Fig. 21) a marked similarity to the  $F_1$  hybrid, *rubra*.



FIGS. 16-26. The nucleolar relationships at prophase and telophase in *Salix* species and hybrids. ( $\times 1,800$ .) Fig. 16, *S. Caprea*, early prophase. Fig. 17, *S. Caprea*, late prophase. Figs. 18 and 19, *S. Caprea*, diploid and tetraploid races respectively at telophase. Fig. 20, *S. Bockii*, late prophase. Fig. 21, *S. daphnoides*, late prophase. Fig. 22, *S. Andersoniana*, mid-prophase. Figs. 23-6, late prophase in *S. lapponum*  $\times$  *aurita* (diploid), *rubra* (triploid), *Caprea*  $\times$  *atrocinerea* ( $F_1$ ) and *Caprea*  $\times$  *atrocinerea* ( $F_2$ ) respectively.

## DISCUSSION

### 1. Chromosomal and nucleolar number.

The base numbers 19 and 22, first reported by Blackburn and Harrison (1924), and verified by others, are shown in this study to apply to a number of *Salix* species not hitherto investigated. No base number other than these

has been found, and no instance of genuine aneuploidy in the Salices has yet been encountered by the writer. Those species with the 22-base are not considered to be aneuploid in the true sense, as they are almost certainly derived by fragmentation from the 19-paired species.

Both 19 and 22 as haploid numbers are comparatively rare in the Plant Kingdom. Reference to Tischler's list (1938) and the Merton Catalogue (Maude, 1939) shows that, so far as is known, few herbaceous genera have either of these basic chromosome numbers. *Brassica Napus* (Howard, 1938) and two *Carex* species (Heilborn, 1924) have the 19-base, whilst only *Scirpus compressus* (Håkansson, 1928) appears to have 22 as its haploid number. There are, however, a number of genera with one or more species possessing 11 as the haploid chromosome number, e.g. *Diplotaxis*, *Circaea*, *Aegopodium*, *Asperula*, *Primula*, *Blackstonia*, *Euphrasia*, *Lemna*, *Iris*, &c. Amongst the woody genera, *Magnolia*, *Liriodendron*, *Vitis*, *Drimys*, *Trochodendron*, and *Cercidiphyllum* have the 19-base, besides *Salix* and *Populus*, and apart from the Salicaceae only *Syringa* has the base of 22.

Reference to the cytological features of other 'Amentiferae' throws little light on the origin of the Salicaceae. In the Fagales, the Betulaceae (Woodworth, 1929, 1931; Jaretsky, 1930) have 7 (*Betula*, *Alnus*) and 8 (*Carpinus*, *Ostrya*, *Ostryopsis*) as their commonest base numbers. The nine species of *Corylus* exhibit a haploid number of 14. No polyploid species are known in the Fagaceae, the base number of 12 being typical of *Quercus*, *Fagus*, and *Castanea*. In the Juglandaceae (Woodworth, 1931) the base number 16 applies to *Juglans* and *Carya*, and in the Myricaceae (Stokes, 1937) the base numbers 8 and 16 are found in *Myrica* and *Comptonia* respectively.

Most of the genera of the Myricaceae, Betulaceae, and Juglandaceae thus possess the base number of 8 or a multiple thereof; and the Fagaceae, with the base number of 12, may easily be imagined to have arisen by simple derivation from the base of 8. The number 19 for the Salicaceae is, however, difficult to derive in a like manner without speculation unjustified by known facts.

Variation in basic chromosome number unaccompanied by variation in systematic characters, a comparatively rare phenomenon in a species, has been recorded for *Salix triandra* (Blackburn and Harrison, 1924). Further examples of this probably occur in the Salices, however, as may be inferred from the curious somatic chromosome number of 41 discovered in one of the *Caprea* × *viminalis* crosses reported in this study. The hybrid in question obviously arose from the fusion of gametes containing respectively 19 and 22 chromosomes. Either parent may have possessed the less common base number, 22, but the likelihood of the *Caprea* parent having this number is suggested by the occurrence of the 22-base in another member of the *Capreae* group, *Salix livida*.

Turning now from the consideration of base number to that of variation in the number of haploid sets, *Salix* is seen to provide one of the few examples of a woody genus with a large number of polyploid species. The range forms



an even series from the diploid to the decaploid condition, with but one example of an odd degree of polyploidy, namely the triploid *S. daphnoides*. Diploidy appears to be characteristic of the Synandreae, a group which, if floral reduction is accepted as the chief criterion of advancement, must be regarded as relatively recent. The Diandreae appear typically to be mostly diploid or tetraploid, with hexaploidy represented in the Phyllicifoliae; and tetraploidy appears to be the stable condition of the pleiandrous willows, including the Albae and the Fragiles, but with the exception of *S. triandra*, which is either diploid or tetraploid.

One of the most striking features associated with polyploidy in this genus is the widespread occurrence of polyploidy within a given species, unaccompanied by taxonomic differences. Until now the only such example in the willows has been that of *Salix aurita*, observed to be both diploid and tetraploid by Harrison (1926) and Håkansson (1929). The present study, however, has brought to light several other such examples. *Salix lapponum* may be either tetraploid or diploid, no systematic differences being discernible between the races; *S. Myrsinites* may similarly be either diploid or decaploid; *S. triandra* may be either diploid or tetraploid on the 22-base, as well as diploid on the 19-base; *S. daphnoides* has both diploid and triploid races not readily distinguished from one another, though there is a strong suggestion that this 'species' is in reality an  $F_1$  hybrid; and, coming to the group in which the occurrence was first reported, *S. Caprea* as well as *S. aurita* may be encountered in either the diploid or tetraploid form.

In general, therefore, it seems that the metabolic plasticity of *Salix* is such that the number of haploid sets or even the basic chromosome number may alter spontaneously, without any corresponding modification of external characters. Polyploidy within the species without change of systematic characters is known in several other cases, notably *Callitriche stagnalis* ( $n = 5$  or 10; Jørgensen, 1923); *Festuca elatior* ( $n = 7, 21$ , or 35; Lewitsky and Kuzmina, 1927); *Silene ciliata* ( $n = 12, 24$ , or 96; Blackburn, 1928); and *Crepis bungei* ( $n = 4$  or 8; Hollingshead and Babcock, 1930). Notwithstanding that this may be considered by some to impair to some extent the value of polyploidy as a criterion of phylogeny in the Salices, it nevertheless remains true that diploids appear to be stable in the Synandreae, tetraploids in the Pleiandreae, and an assortment of both diploids and tetraploids in the Diandreae, with both in certain of the species.

It may be significant that the plants showing the highest degree of polyploidy (*glauca*, octoploid on the 22-base, and *Myrsinites*, decaploid on the 19-base) originated from a northerly station, Oslo. As is now well known, polyploidy may be induced artificially by heat or cold treatment. Thus Shimotomai (1927) reported the formation of both diploid and tetraploid pollen grains in *Scilla* and *Liriope* subjected to a temperature of  $0^{\circ}\text{C}.$ , and Washiashi (1935) has obtained similar results with *Disporum*, whilst Müntzing and his co-workers (1936) have induced tetraploidy in barley by both heat and cold treatment. There are many other well-known instances of this

type of behaviour. It seems reasonable to suppose that the high degrees of polyploidy in *Salix Myrsinites* and *S. glauca* may represent a reaction to a cold environment. The same species from more southerly situations have already been reported as diploids (Blackburn and Harrison, 1924). A parallel exists in the case of *Rosa acicularis*, which is normally diploid (Blackburn and Harrison, 1921), but has also an octoploid race in northerly stations in the United States (Erlanson, 1929), apparently without change in systematic characters. Since in these instances the phenotype is apparently not affected by even drastic alteration in the number of haploid complements, marked variation in the number of haploid sets is probably incidental and not of any great importance in evolution.

It is interesting to consider nucleolar number (which corresponds with the number of satellited chromosomes) in relation to polyploidy in *Salix*. Whereas in some genera with the species forming a polyploid series (see the critical review of Gates 1942) the nucleolar number for a given species broadly conforms with the degree of polyploidy, as in *Triticum* (Pathak, 1940), this is apparently not so in *Salix*. Except in the case of the synandrous species, which have a maximum of 2 nucleoli, the diploids have 4 nucleoli; the tetraploids (except *Salix alba* var. *caerulea*) likewise have 4; the hexaploid *Andersoniana* has 8; and the respectively octoploid and decaploid races of *glauca* and *Myrsinites* both have 12 or more. The nucleolar number during mitosis and the high base number lend strong support to the view that the normal diploids are secondary polyploids.

## 2. *Chromosome morphology.*

Reference to the drawings of somatic complements from the representative *Salix* species emphasizes the remarkable uniformity in chromosome size and type. The great majority of chromosomes are alike in morphology in all species; the differences between complements of species with the same chromosome number are inconspicuous, and indeed are detectable beyond reasonable doubt only after prolonged and detailed study.

In spite of this lack of prominent structural variation, certain chromosome types such as those with secondary constrictions, those without any apparent constriction, and those which are satellited, appear to have taxonomic importance. Whatever the species, the majority of the somatic chromosomes have median constrictions, approximately central in position. Usually, certain of the longest chromosomes have secondary constrictions, the two constriction regions dividing them into three approximately equal segments. As a rule there are some short chromosomes present without any apparent constriction region, and there are always some satellited chromosomes. The satellites are on relatively long stalks, almost invisible, and take the form of exceedingly minute but sharply defined spheres.

Reference to Table I shows that the 19- and 38-paired species of the *Diandrae* and *Pleiandrae* maintain a surprisingly constant idiogram. There appear always to be 4 long chromosomes with secondary constrictions;

4 satellited chromosomes, 2 with median constrictions and a further 2 either constricted or unconstricted; and generally 4 to 8 unconstricted chromosomes in the shortest length-class.

Compared with most Angiosperms, the willows have apparently been evolving for a very long period, records of their occurrence having been obtained as far back as the Lower Cretaceous formations (the occurrence of fossil Salicaceae is conveniently summarized by Berry, 1923). As already seen, the exceptionally high base and nucleolar numbers support the view that secondary polyploidy has played some part in the evolution of the karyotype. In addition fragmentation, which satisfactorily accounts for the derivation of the 22-base from the 19-base in present-day species, may well have been another of the mechanisms by which successive increases in base number have been achieved during karyophylaxis. A cleavage of the long chromosomes with secondary constrictions into either three unconstricted members, or one unconstricted member and one with a median constriction, is readily visualized; and combinations of these two methods within the same nucleus might readily have given rise to other transitional base numbers during the course of evolution. If this is so, it seems very probable that those existing species which possess the highest number of secondarily constricted chromosomes are the oldest.

The synandrous willows so far studied (*S. purpurea*, *Bockii*, *hypoleuca*) all have 19-paired complements, and are distinguished from the typical diploid diandrous species by the possession of only two long chromosomes with secondary constrictions, and two (instead of four) with satellites. Applying the above argument, the evidence of chromosome morphology suggests that they are of more recent origin. This agrees with conclusions reached by considering the reduction of floral parts, the relatively low number of species, and the comparatively restricted geographical distribution.

From this point of view the Fragiles and Albae, which exhibit the presence to a varying extent of chromosomes of the secondarily constricted type (see Wilkinson, 1941), must also be relatively recent. Their strongly marked tendency towards variability in the number of floral parts, which has been responsible for uncertainty on the part of taxonomists as to whether these groups ought to be included in the Diandrae or the Pleiandrae, taken in conjunction with the cytological evidence, reveals them in the writer's view as transitional groups, possibly linking the primitive Pleiandrae with the more advanced Synandrae.

A remarkable feature of the somatic complement of those tetraploid species which have diploid strains (e.g. *Caprea*, *aurita*, *lapponum*) is that the chromosomal aggregate of the tetraploid strain, from the point of view of chromosome morphology, is not precisely double that of the diploid. Thus, in the tetraploid, the number and type of secondarily constricted chromosomes and of satellited chromosomes is the same as in the diploid. Since the aggregate of genes does not appear to be altered when the tetraploid arises (no systematic differences are observable), one is forced to conclude that structural rearrange-

ment has accompanied the doubling of the chromosome complement, resulting in the numerical reduction of the long chromosomes with secondary constrictions. This is to some extent consistent with the occurrence in the tetraploid of less than the expected number of unconstricted chromosomes (6 instead of 8). It must also be true for the other tetraploid species normally found, in view of their presumed derivation from ancestral diploids with the same number of secondarily constricted chromosomes, whether they have arisen as autopolyploids or, what is much more likely in view of the readiness with which hybridization goes on in the *Salices*, as amphidiploids. In either case the diploid originals will usually have had the typical diandrous complement.

Particular interest centres around the question of the satellited chromosomes in willow complements, especially in view of the occurrence of amphiplastic effects (Navashin, 1934), and the recent further clarification of nucleolar relations (see Gates, 1942). Most pleiandrous and diandrous complements, whether diploid or tetraploid, have two pairs of satellited chromosomes, whilst the synandrous complements so far studied have only one pair. As has been indicated elsewhere by the writer (Wilkinson, 1941), a mechanism which maintains the constancy of satellite number in diploids and tetraploids is provided by assuming an amphidiploid origin for the tetraploid species, and allowing for the fact that 'differential amphiplasty' involving satellite loss may well be a feature attending the setting up of the original hybrid complement from the diploid species before doubling occurs. It is not possible to say at present how satellite modification may have occurred in the tetraploid strains of species which are normally diploid (e.g. *Caprea aurita*); here hybridity cannot have taken any part in the modification, and as yet none but a purely speculative explanation can be offered.

In considering the origin of the bi-satellited tetraploid somatic complement of the cricket-bat willow, the writer (1941) offered as a suggestion the former existence of an ancestral diploid with only two satellites. This is now seen to be further supported by the fact that the 19-paired complements of the synandrous species studied have only one satellited pair of chromosomes, and these species may likewise have descended from such an ancestral type. During karyophytesis secondary polyploidy involving duplication of the satellited pair may have led to the setting up of the typical diandrous karyotype, whilst in the formation of the synandrous complement the satellited pair of ancestral chromosomes may not have been involved. An alternative explanation for the derivation of the synandrous karyotype emerges from the suggestion of Gates (1937), that mutation has occasionally resulted in the reduction of satellite number in secondary polyploids. On this basis, the somatic complement of the *Synandrae* has arisen by mutation from a diandrous type, and is therefore relatively advanced.

'Differential amphiplasty', involving satellite loss, has clearly occurred in the case of the  $F_1$  interspecific hybrids dealt with in this study. In all cases only two satellited chromosomes appear in the metaphase complement of the

hybrid, and this is verified by study of the nucleolar number at prophase and telophase. In those crosses where there were two satellited chromosomes in each of the respective parental contributions, it is impossible to name the parent in which the satellites were suppressed; in the three cases where *S. purpurea* was one of the parents, however, the single satellite in the *purpurea* haploid complement was obviously suppressed. In a population of  $F_2$  segregates from a cross between tetraploid *Caprea* and *atrocinerea* parents occurring naturally in Gower, four satellited chromosomes were found on the metaphase plates and four nucleoli at early telophase during mitosis. Thus the amphiplastic effect involving satellites does not appear to extend beyond the first hybrid generation.

### 3. Chromosome size.

In common with most of the woody dicotyledonous genera (see Stebbins, 1938) the size of *Salix* chromosomes is very small, and similar for all species examined. The longest does not exceed  $1.75 \mu$ , and the shortest for a pure species is  $\frac{1}{2}-\frac{3}{4} \mu$ . In some  $F_1$  hybrids the shortest chromosomes are  $\frac{1}{4}-\frac{1}{2} \mu$  long. For reasons fully dealt with in a previous study (1941) the writer, in considering chromosome size, has resorted to an allocation of the chromosomes into length-classes.

TABLE III. *Salix Species and Hybrids*  
Typical Assortment of Chromosome Numbers into Length-classes.

| Type.  | Microns. |          |          |          |          |          |
|--|----------|----------|----------|----------|----------|----------|
|  | 0.25-0.5 | 0.5-0.75 | 0.75-1.0 | 1.0-1.25 | 1.25-1.5 | 1.5-1.75 |
| Diploid $2n = 38$ (e.g. <i>viminialis</i> )              | —        | 8        | 12       | 10       | 4        | 4        |
| Diploid $2n = 44$ (e.g. <i>cordata</i> )                 | —        | 16       | 12       | 10       | 4        | 2        |
| Tetraploid $2n = 76$ (e.g. <i>lapporum</i> )             | —        | 12       | 28       | 24       | 8        | 4        |
| Tetraploid $2n = 88$ (e.g. <i>phylicifolia</i> )         | —        | 22       | 28       | 26       | 8        | 4        |
| HYBRIDS  |          |          |          |          |          |          |
| <i>lapporum</i> $\times$ <i>viminialis</i> ( $2n = 38$ ) | —        | 10       | 10       | 10       | 4        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>herbacea</i> $\times$ <i>lapporum</i> ( $2n = 38$ )   | 2        | 10       | 10       | 8        | 4        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>lapporum</i> $\times$ <i>Caprea</i> ( $2n = 38$ )     | 2        | 12       | 8        | 6        | 6        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>lapporum</i> $\times$ <i>aurita</i> ( $2n = 38$ )     | 2        | 12       | 8        | 6        | 6        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>Caprea</i> $\times$ <i>viminialis</i> ( $2n = 41$ )   | 2        | 13       | 10       | 6        | 6        | 4        |
| Expectation  | —        | 12       | 12       | 10       | 4        | 3        |
| <i>aurita</i> $\times$ <i>atrocinerea</i> ( $2n = 76$ )  | —        | 16       | 26       | 18       | 12       | 4        |
| Expectation  | —        | 12       | 28       | 24       | 8        | 4        |
| <i>purpurea</i> $\times$ <i>viminialis</i> ( $2n = 38$ ) | 4        | 6        | 8        | 8        | 8        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>triploid rubra</i> ( $2n = 57$ )                      | 2        | 12       | 18       | 15       | 6        | 4        |
| Expectation  | —        | 10       | 20       | 17       | 6        | 4        |
| <i>purpurea</i> $\times$ <i>aurita</i> ( $2n = 38$ )     | 2        | 8        | 8        | 8        | 8        | 4        |
| Expectation  | —        | 8        | 12       | 10       | 4        | 4        |
| <i>aurita</i> $\times$ <i>phylicifolia</i> ( $2n = 63$ ) | —        | 17       | 20       | 16       | 6        | 4        |
| Expectation  | —        | 15       | 20       | 18       | 6        | 4        |

Measurement of the chromosomes of the species and hybrids described in this study has been undertaken for not less than twelve good metaphase plates of each. Typical results are tabulated in a condensed form in Tables III and IV.

TABLE IV  
*Salix Caprea* × *atrocinerea* Colony

Typical Assortment of Chromosome Numbers into Length-classes.

|        |  | Microns. |          |          |          |           |           |
|--------|--|----------|----------|----------|----------|-----------|-----------|
|        |  | 0.25-0.5 | 0.5-0.75 | 0.75-1.0 | 1.0-1.25 | 1.25-1.50 | 1.50-1.75 |
| Plant. |  |          |          |          |          |           |           |
| I      | <i>Salix Caprea</i> parent ( $2n = 76$ )                         | —        | 12       | 28       | 24       | 8         | 4         |
| II     | „ <i>Caprea</i> × <i>atrocinerea</i> F <sub>2</sub> matroclinous | —        | 12       | 28       | 24       | 8         | 4         |
| III    | „ „ „ „  | —        | 10       | 30       | 24       | 8         | 4         |
| IV     | „ „ „ „  | —        | 12       | 28       | 24       | 8         | 4         |
| V      | „ „ „ „  | —        | 12       | 26       | 26       | 8         | 4         |
| VI     | „ „ „ F <sub>1</sub>   | 4        | 12       | 26       | 18       | 12        | 4         |
| VII    | „ „ „ F <sub>2</sub>   | —        | 12       | 28       | 24       | 8         | 4         |
| VIII   | „ „ „ „  | —        | 12       | 28       | 24       | 8         | 4         |
| IX     | „ „ „ „  | —        | 10       | 28       | 26       | 8         | 4         |
| X      | „ „ „ „ patroclinous   | —        | 12       | 28       | 24       | 8         | 4         |
| XI     | „ <i>atrocinerea</i> parent ( $2n = 76$ )                        | —        | 12       | 28       | 24       | 8         | 4         |

The numbers of chromosomes in various length-classes for the hybrids tend to deviate in varying degree from expectation, and the deviation may be explained on the basis of 'neutral amphiplasty', described by Navashin in his work on *Crepis* (1934). In that paper the somatic complements of the hybrids *capillaris* × *tectorum*, *capillaris* × *neglecta*, and *capillaris* × *aspera* were the ones chiefly considered from this point of view. On the scheme of taxonomy given for *Crepis* by Hollingshead and Babcock (1930), the species *capillaris*, *tectorum*, and *neglecta* belong to the subgenus *Eucrepis*, and *aspera* to the subgenus *Barkhausia*. The degree to which one parental haploid set 'moulds' the other does not vary much whichever way the cross is taken.

Even during the preliminary inspection of the somatic metaphase plates of the willow hybrids here described, the writer was impressed by the fact that for some crosses (e.g. *purpurea* × *viminalis*, *Caprea* × *viminalis*) unusually long and unusually short chromosomes were assorted together, whilst in others (e.g. *aurita* × *atrocinerea*, *laponum* × *viminalis*), the concurrence of long and short chromosomes was not so noticeable. Reference to Table III, showing the assortment of the chromosomes in various length-classes, reveals the fact that different hybrids appear to exhibit varying degrees of 'neutral amphiplasty'. Thus, hybrids between species in the same or nearly related groups on Linton's modification of Andersson's systematic scheme (e.g. *aurita* × *atrocinerea*, *laponum* × *viminalis*) show a relatively low degree of alteration of chromosome length, whilst those between species further removed, such as *purpurea* × *viminalis*, *laponum* × *aurita*, show a greater degree of neutral amphiplasty.

The inherent unsuitability of the material militates against a more exhaustive analysis of the situation from this point of view. The correlation between the extent of the amphiplastic effect and taxonomic divergence may well be a profitable line of inquiry in cytotaxonomy, particularly if applied to the study of a genus with a low number of long and easily measurable chromosomes, and with the ability to form hybrids between species generally accepted as widely separated in taxonomy.

A convenient opportunity for studying the transmission of amphiplasty to the  $F_2$  generation has arisen whilst investigating the taxonomy of the *Salices* of Gower. In this area there are several localities where *S. Caprea* and *S. atrocinerea* var. *aquatica* occur close together, with  $F_1$  and a large range of  $F_2$  segregates nearby. Reference to Table IV, which records the results of cytological examination of a representative range of types from one such colony, shows that whereas differential and neutral amphiplasty are clearly apparent in the  $F_1$  generation, no trace of either of these effects appears to be transmitted to the  $F_2$  segregates.

#### 4. *Concluding remarks.*

The results of this cytological examination are not offered as affording any critical elucidation of the evolution of the *Salices*. It may, however, serve a useful purpose in giving further general support to so well-trying a taxonomic classification as that of Andersson, and to such views on the relationship between phylogeny and floral characters as are held by Fisher. It further adds more weight to the evidence, the accumulation of which began with the inquiries of Blackburn and Harrison, for the karyophytesis of *Salix* by amphidiploidy, secondary polyploidy, fragmentation, and probably mutation. Another promising line of investigation has been opened by the application of Navashin's views on 'amphiplasty' to the analysis of the karyotype of inter-specific hybrids in this genus. As has been seen, there are indications that the extent to which the presence of one of the haploid sets of chromosomes in a first generation hybrid influences the other depends upon the nearness of the parental species to one another as judged by taxonomic standards; and further work may well justify the adoption of amphiplasty as yet one more criterion in the species concept.

#### SUMMARY

1. The chief object of this inquiry has been to gather further information on the karyology of *Salix* and to discover how far this elucidates the phylogeny of the genus.

2. Several new chromosome numbers are recorded on both base numbers (19 and 22), ranging from *S. Bockii*, which is diploid on a base of 19, to *S. glauca*, which is octoploid on the 22-base, and *S. Myrsinites*, here found to be decaploid on the 19-base.

3. Further instances have been discovered of polyploidy within the species

unaccompanied by systematic differences, e.g. *S. lapponum* (diploid and tetraploid), *S. Myrsinites* (diploid and decaploid).

4. Certain small but critical differences have been detected between the chromosome complements of synandrous willows and those of other groups. These involve satellite number and the number of chromosomes with secondary constrictions.

5. The cytological evidence supports the view held by workers in other fields that the Synandreae are the most recent of the willows.

6. Nucleolar number does not correspond with degree of polyploidy in the Salices.

7. Differential and neutral amphiplasty occur in first-generation hybrids of *Salix*.

8. The extent to which neutral amphiplasty is manifest appears to depend upon the degree of separation of the species on a widely accepted systematic scheme such as that of Andersson.

9. Amphiplastic effects are apparently not transmitted to the second hybrid generation.

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# Studies in the Vernalisation of Cereals

## VIII. The Role of Carbohydrate and Nitrogen Supply in the Vernalisation of Excised Embryos of 'Petkus' Winter Rye

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With four Figures in the Text

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### INTRODUCTION

**I**N a previous paper of this series (Gregory and Purvis, 1938) it was shown that the excised embryo of rye when supplied with nutrient salts and glucose responded to vernalisation treatment in the same manner as the whole grain and to a comparable degree. Similar results had previously been reported by the same authors (1936), by Konovalov (1937), and by Bassarskaja (1941) who records the work of I. M. Drozzin in 1936. The locus of vernalisation would then appear to lie within the embryo as postulated by Lyssenko

(1932). Indeed, later work (Purvis, 1940) has demonstrated that the isolated stem apex of the embryo, if supplied with sugar and submitted to low temperature, regenerates the whole plant, which shows itself to have been vernalised. A similar result is recorded by McKinney and Sando (1935) for embryos mutilated before chilling but maintained in contact with the endosperm. Gregory and Ropp (1938) have further shown that if sugar is omitted from the medium the embryos remain unvernalsed.

From these experimental results the conclusion was drawn that the large accumulation of the hormone 'blastinin' found by Cholodny (1936) in the endosperm of maize does not play an essential part in the vernalisation process, and that the embryo is capable of synthesizing any hormones that may be necessary for vernalisation from external sources of carbohydrate in the presence of inorganic salts, with possibly the aid of additional substances already present in the embryo before imbibition. It will be shown in this paper that embryos excised from *unsoaked* seed can be vernalised, so that the objection to the excised embryo technique raised by Cholodny (1939) and cited by Whyte (1939) is not valid. The factors which can possibly participate in the vernalisation process are (i) the embryo and its constituents; (ii) the source of organic carbon, (iii) the source of nitrogen and the mineral salts, and (iv) the effect of low temperature. In order to throw light on the role of carbohydrates and of nitrogen in vernalisation, a comprehensive series of experiments was carried out in 1939, in which excised embryos were subjected to a low temperature for 6 weeks on agar media containing varying proportions of these nutrients. The part played by nitrogen was studied by using media with a range of concentrations of nitrate or of equivalent amounts of organic nitrogen compounds or ammonium salts. In the same way attempts were made to discover the relative value of different carbohydrates as well as the critical concentration of sucrose at which vernalisation was possible and also, by applying or withholding sugar at different stages of the treatment, to determine if vernalisation proceeded at a uniform rate throughout the period of treatment.

The results of these experiments were not conclusive; in the first place, on media containing little or no sucrose some degree of vernalisation was manifest, and secondly, this applied only to a few individual embryos. Thus in such series the flowering date was so highly variable that any precise assessment of the vernalising power of a particular treatment was impossible.

To ensure the precision of further work it was necessary to find a method of eliminating this variability. It appeared that quite small amounts of sucrose were adequate for vernalisation, and therefore it seemed probable that in those individuals which had become vernalised on sugarless media there had in fact been available the small amount of sugar requisite for the process. Since the medium was uniform for any series the source of this sugar must have been within the individual embryo, either as stored sugars and starch in the tissues or as starch grains adhering to the scutellum. Experiment showed that at room temperature some embryo growth occurred on sugar-

free media, even when agar, suspected as a possible source of carbohydrate, was replaced by glass-wool. This fact suggested a method of getting rid of such 'residual' carbohydrate. Embryos used for 1940 experiments were therefore prepared for vernalisation after excision by growing them on sugarless medium at room temperature for 4 days, by which time 'residual' growth was practically complete. Periods of starvation even as great as 28 days did not kill all the embryos, and the relation of this 'residual' growth to later growth and vernalisation was studied during 1940. This treatment successfully reduced the variability of the low sugar series, but the results raised a number of other problems which required further experiment, and therefore discussion of these experiments with 'residual growth' will be relegated to a later communication.

In 1939 the effect of organic compounds other than sucrose was also studied. It was known from earlier work that glucose was effective for vernalisation. The normal substratum of the cereal embryo consists, of course, of a sugar complex resulting from starch hydrolysis, with accessory substances in addition, the importance of which has been emphasized by Schander (1934). Dragone-Testi (1935) investigated the value of various carbohydrates for the growth of wheat and maize embryos excised without soaking. The embryos were germinated at room temperature in light for 5 days on 1.5 per cent. agar containing mineral salts alone or with the addition of 1 per cent. of the substance in question. No numerical data were presented. With wheat best growth and survival to fruiting resulted from the use of sucrose, fructose, and glucose. Maltose, lactose, glycerol, arabinose, and galactose allowed some embryo growth, but mannose was little better than minerals alone. Only embryos grown on the three sugars first named survived planting out. Of more complex carbohydrates, raffinose and inulin gave positive growth. Dragone-Testi attributes great importance to the presence of fructo-furanose groups in the molecule; this would account for the value of sucrose and possibly for the superiority of sucrose over fructose itself, since the latter would probably exist in the medium as the pyranose form; it would also account for the utilization of raffinose and inulin by the embryo. The value of other substances named cannot be explained in this way.

The substances used in the vernalisation experiments of 1939 include among carbohydrates pentoses, hexoses, disaccharides, trisaccharides, and polysaccharides, as well as alcohols and organic acids. Their value in vernalisation proved to be as variable as their utility for growth; unfortunately the high variability discussed above appeared also in series germinated on substances of low vernalising power. The results as they stand do, however, throw some light on the relative efficiency for growth and for vernalisation of a range of carbohydrates and allied substances.

In addition the effect of the hormones heteroauxin and aneurin, either singly or together, was also studied both in the presence and absence of sucrose, and their effect at 20° C. was also tested without vernalisation treatment. These substances tended to depress growth and in relatively

high concentrations produced distortion of the embryos; corresponding reductions in vernalisation were also noted. Further, in the absence of sugar, or of low-temperature treatment, no vernalising effect was noted. An attempt was also made to 'vernalise' the winter rye embryos by germination at room temperature on media containing extracts of spring rye embryos. In making these extracts a bacterial filter was used to avoid the possible destruction of vernalising substances by heat. The plants, however, remained in the vegetative condition for a period of over 12 weeks, after which the experiment was discontinued.

The scope of this paper is confined to the consideration of the effect on vernalisation of varying the carbohydrate and nitrogen supply, and to a discussion of the results obtained within the limits imposed by the unforeseen difficulties of the experiment.

### EXPERIMENTAL METHODS

#### 1. *Scope of the experiments.*

In a control series embryos were grown on the standard medium containing 2 per cent. sucrose and 2 millimols of nitrogen as calcium and potassium nitrates, and the period of vernalisation was varied from 1 to 6 weeks. The purpose of this set was to provide a graded series to serve as basis for assessing the vernalising power of the other media used. The following experimental series are described in this paper:

- (i) Nitrogen, supplied as nitrate ranging from 2 mmol through 0.2, 0.04, 0.02, 0.002 mmol to nil.
- (ii) Nitrate nitrogen replaced by ammonium sulphate, asparagin, urea, and glycine in amounts containing 2 mmol of nitrogen.
- (iii) Sucrose used in the following percentage concentrations: 4, 0.5, 0.25, 0.125, 0.0125, 0.00125, and controls without sugar.
- (iv) The embryos were transferred to media containing either 2, 0.125, or 0.0125 per cent. sucrose after periods of 1, 2, 3, 4, or 5 weeks on a sugar-free medium at 2° C.
- (v) After preliminary treatment with sucrose at the three concentrations named in (iv), this nutrient was withheld during the last 1–5 weeks of vernalisation treatment.
- (vi) Sucrose was replaced by equivalent amounts of the following:
  - Pentose sugars: ribose,<sup>1</sup> arabinose, xylose.
  - Monohexose sugars: glucose, fructose, galactose, mannose.
  - Disaccharides: maltose.
  - Trisaccharides: raffinose.
  - Polysaccharides: fructosan,<sup>1</sup> from barley leaves.
  - Alcohols: glycerol, mannitol.
  - Acids (as sodium salts): malic, pyruvic, succinic.

<sup>1</sup> The author is indebted to Messrs. Roche Products Ltd. for the sample of *d*-ribose, and to Dr. H. K. Archbold for the fructosan isolated by her from barley leaves (Archbold and Barter, 1935).

In a second series of experiments performed in 1940 attention was mainly directed to the elimination of the residual sugar in the embryos before vernalisation. To this end growth at 20° C. on sugarless medium preceded the vernalisation treatment. These experiments will be described in a subsequent communication.

## 2. *Preparation of media.*

The standard medium, a modification of that used by White (1934) for the growth of excised root-tips, was prepared as follows:

|  |       |                |
|--|-------|----------------|
| Calcium nitrate . . . . .                | 0.6   | mmol per litre |
| Magnesium sulphate . . . . .             | 0.3   | " "            |
| Potassium nitrate . . . . .              | 0.8   | " "            |
| Potassium chloride . . . . .             | 0.87  | " "            |
| Potassium dihydrogen phosphate . . . . . | 0.09  | " "            |
| Ferrous sulphate . . . . .               | 0.003 | " "            |
| Sucrose . . . . .                        | 2%    |                |
| Agar . . . . .                           | 0.7%  |                |

Slants were prepared in test-tubes each containing 7 ml. of this medium, and autoclaved for 15 minutes at 120° C.

It was thought that the hydrolysis of the less stable sugars in experiment (vi) might be avoided by sterilizing the media on three successive days for 30 minutes at 100° C. instead of autoclaving at 120° C. A few tubes of some of the media concerned, damaged by accident, were replaced by autoclaved tubes with no apparent effect on the growth of the embryos or in the behaviour of the resulting plants. It appears, moreover, that hydrolysis may be more marked during successive steamings at 100° C. than during autoclaving (Armstrong and Armstrong, 1934, p. 50). In critical experiments of this nature the use of bacterial filtration methods would be desirable.

The pH value of the media was not specially adjusted, as it was found to be fairly constant in the region of pH 7.5. At this level changes in the medium during sterilization might be assumed to be small. In media with reduced nitrate content, potassium sulphate and calcium chloride were used to adjust the concentration of the metallic ions.

## 3. *Treatment of embryos and plants.*

Petkus winter rye was used throughout these experiments as in previous work. After soaking for 3–4 hours each grain was sterilized by immersion in 80 per cent. alcohol for 5 seconds, and after drying on sterilized filter-paper transferred to a flamed slide. The embryo was uncovered and removed with a succession of flamed, spear-shaped needles under a low-power dissecting microscope. In the experiments of 1939 contamination of the cultures by bacteria or fungi was practically absent, but in 1940 much more trouble was encountered. As the number of embryos excised to obtain the desired number of plants was based on the experience of the previous year, this trouble with fungi had an unfortunate effect in reducing replication in the second year.

In 1939 vernalisation was carried out in a refrigerator with the temperature

automatically controlled, averaging 2° C. and ranging from just below 1.5° C. to just over 2.5° C. In 1940 use was made of a constant temperature room with a temperature varying little from 1° C. Except where otherwise stated, the vernalisation period was 42 days.

Though growth on the different media employed varied greatly, nearly all embryos which escaped fungal infection were alive at the end of the period of vernalisation. The stage of planting out in sand proved, however, to be critical, and many embryos which had germinated weakly failed to survive. The perfect technique for dealing with embryos at this stage has not yet been devised. Before the tubes were opened they were kept indoors at room temperature to avoid the harmful effect of strong sunlight on the seedlings which had germinated in the dark, and also to allow increase in their size before handling. This stage, however, must not be too prolonged, as the high humidity in the tubes renders the first leaf very liable to later desiccation. On media with little sugar, growth during vernalisation failed to establish the root system or to produce a leaf capable of photosynthesis. In these cases it was necessary to add sterilized 2 per cent. sucrose solution in sufficient quantity to cover the roots. It is unfortunate that during this post-vernalisation stage all plants could not receive the same treatment, but such variations at room temperatures could not affect vernalisation; they do, however, vitiate any observations on the vegetative growth of the seedlings immediately after treatment.

The plants were grown to maturity out of doors in 12-in. unglazed pots, and nutrients added as described in earlier papers. Throughout the summer a check was kept on the rate of leaf production on the main axis. In this way it was possible to note if the main stem apex survived; where this was destroyed by frit-fly the plant was rejected.

#### *4. Growth measurements before and during the vernalisation period.*

Vernalisation is known to be more effective when some growth is taking place (Lojkin, 1936) and growth is limited in media containing little sugar. It is clearly of interest to determine how far the failure of vernalisation on such media is merely a consequence of inhibited growth; for this reason embryo growth during vernalisation was measured. On removal from the low-temperature chamber the outlines of the embryos were immediately traced on paper. These tracings were made by means of a camera lucida used without a microscope; they form a permanent record (Figs. 3 and 4, pp. 309, 310) and when measured by means of a calibrated map-measurer give an approximate record of embryo size.

#### *5. Presentation of results.*

The variable response to incomplete vernalisation introduces difficulties in the quantitative presentation of results, since a single series may include plants already past anthesis, as well as others in which the ear is still included within the leaf sheaths and its condition of development thus not open to

direct observation. Two methods of dealing with this situation have been previously employed: a graphical method (Purvis and Gregory, 1937) and a method of scoring based on dissections of the growing point, whereby numerical values assessing the stage of maturity reached could be obtained for plants at any stage of the developmental cycle (Gregory and Purvis, 1938). In the 1939 experiment here described the use of the second method had been planned, but the necessary dissections could not be undertaken. Perforce therefore only the external condition could be noted in those plants which had not flowered in 120 days. On this basis, assuming that the completely unvernalsed plants then in the 'rosette' stage would have flowered in 150 days, estimates of the probable time to anthesis of intermediate plants could be made. The results therefore here presented suffer from some uncertainty, which limits the possibility of comparison of the results with those of other years, and makes unavoidable some vagueness in the estimated days to anthesis given later in Tables II and VIII, when short periods of vernalisation are under consideration. In 1940 dissections were carried out, and the 'scoring' method could be employed. To facilitate comparison, however, the 1940 results presented in this paper are calculated by the method employed in 1939.

#### EXPERIMENTAL RESULTS

##### 1. *Vernalisation of embryos excised from dry grain.*

At this point the possible effect of soaking the grain before excision may be considered. Whyte (1939) and Cholodny (1936, 1939) maintain that the hormones stored in the endosperm are largely concerned in the process of vernalisation, and that these may be transferred to the embryo in large amounts during a quite short period of soaking; Schander (1934) has presented evidence that this occurs in the case of substances essential to germination which are stored in the aleurone layer of the grain. This aspect of the technique employing excised embryos was considered by Gregory and Purvis (1938, p. 247), who point out that in their own experiments both vernalised and control embryos were excised from grain soaked at room temperature, so that both were alike in their hormone content at the beginning of germination. Thus, if 'blastinin' is transferred to the embryo during soaking and plays a part in the vernalisation process, the nature of the reaction must be determined by temperature conditions acting on the hormone, and not by the presence or absence of the hormone. In Cholodny's later paper (1939) dealing with this hypothesis, however, he postulates such a temperature effect; namely that the slow growth rate during low-temperature treatment ensures a copious supply of hormone remaining in contact with the meristem, and that this prolonged contact brings about changes in the cells which lead to early flowering. If in 3 hours sufficient hormone can be transferred to produce a concentration at the meristem adequate for this action, which is possible, then the validity of the conclusions as to their non-participation in vernalisation based on the behaviour of embryos excised after soaking might



well be questioned. At the same time the experiment of Gregory and Ropp (1939) shows conclusively that a supply of carbohydrate is also requisite for complete vernalisation. It is exceedingly difficult to excise without injury embryos from dry, ripe grain in the large number required for a comprehensive experiment; in order, however, to assess the possible effect of soaking, fourteen winter rye embryos were excised without previous soaking and germinated on the standard medium at 1° C. Of these two did not survive. A comparison of the behaviour of the twelve remaining embryos with those soaked for 3 hours and vernalised in presence and absence of sugar is given in Table I.

TABLE I

*Effect of Excision from Dry and from Soaked Grain on Vernalisation and Growth of Embryos*

| Treatment.                                   | No. of replicates. | Mean days to anthesis. | Earliest. | Latest. | Coleoptile length (mm.). | Primary root length (mm.). | Total root length (mm.). |
|--|--------------------|------------------------|-----------|---------|--------------------------|----------------------------|--------------------------|
| 1. Excised dry: 2% sucrose.                  | 12                 | 94.0±6.0               | 74        | 145     | 6.3±0.30                 | 6.8±1.3                    | 11.4±2.0                 |
| 2. Excised after soaking 3 hr.: 2% sucrose.  | 10                 | 73.7±2.4               | 66        | 89      | 8.9±0.53                 | 7.8±0.75                   | 16.0±2.1                 |
| Difference (1-2).                            | —                  | 20.3±7.0               | —         | —       | 2.6±0.58                 | 1.0±1.5                    | 4.6±2.9                  |
| 3. Excised after soaking 3 hr.: No sucrose.  | 9                  | 118.0±9.4              | 78        | 150     | 3.5±0.30                 | 1.3±0.12                   | 2.3±0.29                 |
| Difference (1-3).                            | —                  | 24.0±10.7              | —         | —       | 2.8±0.43                 | 5.5±1.5                    | 9.1±2.4                  |
| 4. Excised after soaking 3 hr.: unvernalsed. | —                  | > 150                  | —         | —       | —                        | —                          | —                        |
| Difference (1-4).                            | —                  | 56                     | —         | —       | —                        | —                          | —                        |

The time to anthesis of embryos excised dry and vernalised on 2 per cent. sucrose is at least 8 weeks less than that of unvernalsed plants and is significantly less than that for embryos removed after 3 hours' soaking and vernalised without sugar; it is, however, very significantly greater than that of embryos vernalised on 2 per cent. sucrose after soaking. This lower efficiency of vernalisation in the embryos excised from dry grain may be due to a smaller accumulation of some requisite substances in the embryo, or may simply result from the mechanical injury of the embryo at excision. In any event, *the fact that such embryos can be vernalised at all* proves beyond doubt that should specific hormones be needed for vernalisation they are either present in the dry dormant embryo or can be synthesized by it from simple carbohydrates and inorganic salts. From this it follows that there can be no objection to soaking the grain for a limited period, thus avoiding injury to the embryos and facilitating excision. Further it is evident that the embryo

excised dry differs in no qualitative sense from one that has been soaked in contact with the endosperm.

2. *Growth and vernalisation of excised embryos on standard medium.*

Purvis and Gregory (1937) showed that the degree of vernalisation of whole grain increased with the duration of the low-temperature treatment. In the present work exposure to low temperature on the standard sucrose medium was varied from 1 to 6 weeks, using excised embryos. Embryos were excised and sown on the medium at weekly intervals so that all the treatments ended on the same day, on which day all were measured. Sampling errors thus appear in the measurements obtained. The results for the years 1939 and 1940 are presented in Table II, in which data for growth of coleoptiles and roots during vernalisation are entered, as well as data of flowering. The series for 4 weeks' treatment gave aberrant results both in growth and flowering interval, which, it is suspected, was due to placing the embryos too near the wall of the refrigerator so that they were frozen for a time. This set has been omitted from the Table.

TABLE II

*Effect of Duration of Low-temperature Treatment on Time to Flower and on Growth of the Embryo. Excised Embryos on 2 per cent. Sucrose Medium*

| Expt. of 1939.                  | No. of replicates. | Mean days to anthesis. | Days to earliest anthesis. | Coleoptile length (mm.). | Primary root length (mm.). | Total root length (mm.). |
|---------------------------------|--------------------|------------------------|----------------------------|--------------------------|----------------------------|--------------------------|
| Imbibed embryo before treatment | 11                 | —                      | —                          | 1.7±0.05                 | 1.4±0.05                   | 1.4±0.05                 |
| After 1 week                    | 4                  | >150                   | >150                       | 5.5±0.23                 | 3.0±0.65                   | 3.0±0.65                 |
| „ 2 weeks                       | 4                  | >150                   | >150                       | 6.3±0.52                 | 6.2±1.4                    | 6.6±1.6                  |
| „ 3 „                           | 19                 | 137.9±4.4              | 95                         | 9.4±0.53                 | 11.0±1.1                   | 19.8±2.2                 |
| „ 5 „                           | 8                  | 86.4±2.4               | 75                         | 14.4±1.3                 | 19.9±1.5                   | 47.7±3.4                 |
| „ 6 „                           | 7                  | 76.7±1.4               | 68                         | 20.4±1.9                 | 24.8±3.1                   | 60.9±12.5                |
| Expt. of 1940.                  |                    |                        |                            |                          |                            |                          |
| After 3 weeks                   | 10                 | 150                    | 150                        | 4.2±0.23                 | 5.1±0.85                   | 7.4±1.3                  |
| „ 6 „                           | 7                  | 73.7±2.4               | 66                         | 8.9±0.53                 | 7.8±0.75                   | 16.0±2.1                 |

The lower values for growth of the embryo in 1940 are of interest. The reason does not lie in the sustained lower temperature of 1° C. as compared with the mean temperature of 2° C. in the former year, since in subsequent years embryo growth again attained a level comparable with that in 1939. It must be emphasized, however, that the vernalisation process was not affected by the amount of growth made in the two years, since the time to anthesis required by plants treated for 6 weeks shows good agreement in the two years.

In 1939, 120 days after planting no external evidence of flowering was seen in plants treated for either 1 or 2 weeks; 5 out of 23 plants vernalised for 3 weeks flowered during this 120-day period, and longer treatment progressively decreased the time needed to reach anthesis (Table II). In 1940, none

of the ten plants treated for 3 weeks had in 95 days progressed beyond the stage of 'double ridges' (Gregory and Purvis, 1938). Plants vernalised for 6 weeks, however, flowered in approximately the same number of days as those similarly treated in 1939.

The very slight effect of short periods of treatment is of interest. In earlier work (Purvis and Gregory, 1937) quite short periods of treatment of *whole grain* produced some acceleration in flowering, whereas with *excised embryos* in the experiment under discussion 3 weeks produced very little effect (Fig. 1 p. 301). Further, 6 weeks, the longest period here considered, is less effective in the case of excised embryos than with whole grain.

TABLE III

*Comparison of Time to Anthesis of Whole Grain and Excised Embryos of Vernalised Winter Rye and of Spring Rye. Time to Anthesis in Days*

|                  | Year. | Whole grain. | Excised embryo. | Difference. | P    |
|------------------|-------|--------------|-----------------|-------------|------|
| Spring rye . . . | 1936  | 56.3 ± 0.7   | 65.8 ± 2.6      | 9.5 ± 2.4   | 0.01 |
| Winter rye . . . | 1936  | 82.0 ± 2.1   | 92.2 ± 2.7      | 10.2 ± 3.9  | 0.02 |
| Winter rye . . . | 1940  | 60.5 ± 0.9   | 73.7 ± 2.4      | 13.2 ± 2.4  | 0.01 |
| Winter rye . . . | 1941  | 60.5 ± 0.8   | 75.1 ± 3.8      | 14.6 ± 4.0  | 0.01 |

In Table III is shown the effect of excision on flowering of both spring rye and of vernalised winter rye. In each case the plants grown from excised embryos are significantly later in flowering than those grown in identical conditions from whole grain. With shorter periods of vernalisation this difference is accentuated (Fig. 2). This will be further discussed in § 5.

### 3. *Effect of nitrogen supply.*

The effects of varying the concentration of nitrate in the medium, and of substituting other substances for nitrates as source of nitrogen, are set out in Tables IV and V. There is a slight retardation of flowering where nitrate concentration is reduced, but this is not significant either in any single set or when all the reduced nitrogen series are taken together. Growth of the coleoptiles is also depressed to a similar extent in all series, but the effect barely attains significance.

The effect of nitrate concentration on root growth on the other hand is very marked. A progressive reduction in such growth occurs as the level of the nitrate is increased from nil, and the effect reaches a maximum with a concentration of one-tenth the standard, which is highly significant statistically. At the standard concentration, however, root growth is again increased to a value similar to that of the control series without nitrate.

Replacement of nitrate by other sources of nitrogen was again without effect on flowering; in fact, if the flowering times with all the four substances tested are taken together, the mean agrees precisely with that of the 2 mmol nitrate group. These groups have therefore been included with the standard

TABLE IV  
Effect of Concentration of Nitrate in Medium during Low-temperature Treatment on Vernalisation and Growth of Excised Embryos

| Concn. of nitrate (mmol). | Repli- cates. | Days to anthesis. | Difference. | Length of coleoptile (mm.). | Difference. | Length of primary root (mm.). | Difference. | Length of total roots (mm.). | Difference. |
|---------------------------|---------------|-------------------|-------------|-----------------------------|-------------|-------------------------------|-------------|------------------------------|-------------|
| 2                         | 7             | 76.7±1.3          | —           | 20.4±1.9                    | —           | 24.8±3.1                      | —           | 60.9±12.5                    | —           |
| 0.2                       | 12            | 87.2±4.0          | 10.5±5.3    | 16.6±1.0                    | 3.8±2.0     | 10.3±1.4                      | 14.5±3.0    | 14.6±1.8                     | 46.3±9.6    |
| 0.04                      | 12            | 84.6±3.0          | 7.9±4.1     | 17.5±0.7                    | 2.9±1.7     | 18.2±2.5                      | 6.6±4.1     | 32.5±6.5                     | 28.4±12.7   |
| 0.02                      | 11            | 77.6±1.6          | 0.9±2.2     | 19.0±1.0                    | 1.4±2.0     | 23.4±2.8                      | 1.4±4.3     | 46.6±4.6                     | 14.3±11.4   |
| 0.002                     | 11            | 85.5±5.3          | 8.8±6.7     | 15.3±1.2                    | 5.1±2.2     | 25.0±5.2                      | 0.2±7.0     | 40.1±6.2                     | 20.8±12.6   |
| Nil                       | 12            | 84.3±3.3          | 8.6±4.5     | 18.3±0.7                    | 2.1±1.7     | 29.5±3.1                      | 4.7±4.5     | 60.6±8.2                     | 0.3±8.0     |
| 0.2-0.002 combined        | 46            | 83.9±1.6          | 7.2±4.7     | 17.1±0.5                    | 3.3±1.5     | 19.0±1.8                      | 5.8±4.7     | 33.0±3.1                     | 27.9±9.1    |

TABLE V  
Comparison of Effect on Excised Embryos during Low-temperature Treatment, of Nitrate and of other Sources of Nitrogen with equivalent Amounts (2 mmol N) of Nitrogen

| Source of nitrogen. | Repli- cates. | Days to anthesis. | Difference. | Length of coleoptile (mm.). | Difference. | Length of primary root (mm.). | Difference. | Length of total roots (mm.). | Difference. |
|---------------------|---------------|-------------------|-------------|-----------------------------|-------------|-------------------------------|-------------|------------------------------|-------------|
| Nitrate             | 7             | 76.7±1.3          | —           | 20.4±1.9                    | —           | 24.8±3.1                      | —           | 60.9±12.5                    | —           |
| Ammon. sulph.       | 8             | 73.0±1.3          | 3.7±1.9     | 25.8±6.0                    | 5.4±2.7     | 31.2±2.9                      | 6.4±4.2     | 51.5±7.5                     | 9.4±14.1    |
| Urea                | 9             | 77.8±2.0          | 1.1±2.6     | 24.9±1.0                    | 4.5±2.0     | 40.1±3.9                      | 15.3±5.0    | 62.4±8.0                     | 1.5±14.3    |
| Asparagin           | 9             | 79.2±3.1          | 2.5±3.7     | 20.9±0.8                    | 0.5±1.9     | 30.2±2.1                      | 5.4±3.6     | 53.1±3.7                     | 7.8±11.7    |
| Glycine             | 10            | 76.5±1.9          | 0.2±2.6     | 26.6±0.8                    | 6.2±1.9     | 28.7±4.7                      | 3.9±6.2     | 51.2±7.4                     | 9.7±13.6    |

nitrate group to increase the replication of the control series on 2 per cent. sucrose. The standard medium therefore contains 2 mmol of nitrogen, not necessarily in the form of nitrate. These substances were, on the other hand, more effective for growth than nitrate. Glycine increased coleoptile growth significantly, while an increase due to urea was of doubtful significance. Root growth also was increased in every case, but only in the case of primary roots on urea was the difference significant. The effects of these substances on embryo growth at higher temperatures has not been tested.

TABLE VI

*Time to Flowering of Plants grown from Excised Embryos vernalised  
6 weeks on Media with varying Concentration of Sucrose*

| 1939 experiment           |                  |                      |                    |              | 1940 experiment  |                      |                     |              |   |
|---------------------------|------------------|----------------------|--------------------|--------------|------------------|----------------------|---------------------|--------------|---|
| Sucrose<br>concn.<br>(%). | Repli-<br>cates. | Days to<br>anthesis. | Differences        |              | Repli-<br>cates. | Days to<br>anthesis. | Differences         |              |   |
|                           |                  |                      | from<br>2% sucrose | from<br>nil. |                  |                      | from<br>2% sucrose. | from<br>nil. |   |
| 4                         | 11               | 87.2 ± 6.1           | 10.5 ± 3.6         | 18.4 ± 9.4   | —                | —                    | —                   | —            | — |
| 2                         | 46               | 76.7 ± 1.1           | —                  | 28.8 ± 4.3   | 10               | 73.7 ± 2.4           | —                   | 44.3 ± 9.3   | — |
| 1                         | 10               | 80.4 ± 2.2           | 3.7 ± 2.3          | 25.2 ± 8.6   | —                | —                    | —                   | —            | — |
| 0.5                       | 7                | 80.0 ± 3.4           | 3.3 ± 2.7          | 25.6 ± 10.3  | —                | —                    | —                   | —            | — |
| 0.25                      | 10               | 84.8 ± 2.8           | 8.1 ± 2.4          | 20.8 ± 8.7   | —                | —                    | —                   | —            | — |
| 0.125                     | 10               | 83.5 ± 2.8           | 6.8 ± 2.4          | 22.1 ± 8.7   | —                | —                    | —                   | —            | — |
| 0.01                      | 9                | 92.2 ± 6.6           | 15.5 ± 3.2         | 13.3 ± 9.7   | 10               | 101.6 ± 9.3          | 27.9 ± 9.6          | 16.4 ± 13.3  | — |
| 0.001                     | 6                | 107.2 ± 12.2         | —                  | —            | —                | —                    | —                   | —            | — |
| Nil                       | 10               | 104.6 ± 8.2          | —                  | —            | 9                | 118.0 ± 9.4          | 44.3 ± 9.3          | —            | — |
| Nil & 0.001<br>combined   | 16               | 105.6 ± 6.6          | 28.8 ± 4.3         | —            | —                | —                    | —                   | —            | — |

#### 4. Effect of sucrose concentration during vernalisation.

i. *On flowering behaviour.* The flowering behaviour of plants grown from excised embryos supplied during low-temperature treatment with varying quantities of sucrose is shown in Table VI. The sucrose concentrations used varied from 4 per cent. to nil. The main experiment was performed in 1939, and in 1940 certain of the concentrations were employed in a further test. Comparing the standard concentration (2 per cent.) with lower levels it is seen that the time to anthesis at first slowly increases, and from the column of differences in the table it appears that down to a concentration of 0.5 per cent. no significant differences in flowering are established. Below 0.5 per cent., however, the time to anthesis increases more rapidly and at the same time the differences from the standard treatment become statistically significant, eventually very highly so. Since the series receiving 0.001 per cent. sucrose behaved in flowering very similarly to the control set receiving no sugar, the combined mean has been used as the standard of comparison, thereby increasing the degrees of freedom in the estimation of significance. Increasing the sucrose concentration to 4 per cent. again delays flowering significantly, so that 2 per cent. represents the optimal concentration. Table VI shows that in 1939 the presence of 0.125 per cent. sucrose accelerated flowering by 22 days ( $P < 0.05$ ) while with 2 per cent. sucrose in the medium the acceleration was 29 days ( $P < 0.01$ ). In 1940 an even greater effect, 44 days ( $P = 0.01$ ), was obtained

with 2 per cent. sucrose. There can be no doubt therefore of the importance of a supply of sucrose during the vernalisation treatment; nevertheless the results are not unequivocal. Both in 1939 and in 1940 the variability of the flowering dates becomes much greater as the sugar level is reduced, as may be seen from the standard errors of the mean entered in Table VI. Indeed in the entire absence of external sugar-supply some degree of vernalisation occurred, so that some individual plants reached anthesis. In marked contrast the plants grown from unvernalsed excised embryos germinated on 2 per cent. sucrose showed no sign of flowering at the end of the experiment. This erratic behaviour of individuals leading to great variability makes it difficult to determine with certainty the level of sugar necessary for successful vernalisation. Clearly very small amounts of sucrose suffice to initiate the vernalisation process; the concentration found to be effective, viz. 0.125 per cent., represents only 9 mg. of sucrose in 7 c.c. of the medium supplied to the embryo. That some carbohydrate is stored in the resting embryo in the form of starch may be demonstrated (Brown and Morris, 1890), and to the variable amounts of this supply of carbohydrate has been provisionally attributed the individual variation in response of the excised embryos on a sugar-free medium. Further experiments to elucidate this matter will be presented in a later paper; their general nature has been indicated in the introduction to this paper.

ii. *On the growth of the embryos during vernalisation treatment.* In view of the possible role of growth hormones in vernalisation as postulated by Cholodny (1936, 1939), Melchers (1937), Thimann and Lane (1938), the growth of the embryo during the low-temperature treatment is of interest.

The lengths of the coleoptiles and roots of embryos vernalised for 6 weeks immediately after excision are given in Table VII. On a sugar-free medium

TABLE VII  
*Effect of varying Sucrose Content on Growth of excised Embryos during Vernalisation Treatment*

| Sucrose (%)             | Replicates | Coleoptile (mm.) | Diff. from 2% concn. | Prim. root (mm.) | Diff. from 2% concn. | Total roots (mm.) | Diff. from 2% concn. |
|-------------------------|------------|------------------|----------------------|------------------|----------------------|-------------------|----------------------|
| <i>1939 Experiment</i>  |            |                  |                      |                  |                      |                   |                      |
| 4                       | 11         | 14.9±1.0         | 5.5±2.0              | 20.3±2.4         | 4.5±3.9              | 43.5±7.2          | 17.4±13.4            |
| 2                       | 7          | 20.4±1.9         | —                    | 24.8±3.1         | —                    | 60.9±12.5         | —                    |
| 1                       | 10         | 21.5±1.4         | 1.1±2.3              | 20.8±4.2         | 4.0±6.1              | 42.2±12.4         | 18.7±18.2            |
| 0.5                     | 7          | 19.2±1.5         | 1.2±2.4              | 16.2±4.3         | 8.6±5.3              | 27.5±7.1          | 33.4±28.2            |
| 0.25                    | 10         | 18.8±1.0         | 1.6±2.0              | 8.4±1.5          | 16.4±3.2             | 11.0±2.2          | 49.9±10.7            |
| 0.125                   | 10         | 16.0±0.9         | 4.4±2.0              | 7.6±1.5          | 17.8±3.2             | 7.6±1.5           | 53.3±10.5            |
| 0.01                    | 9          | 6.9±0.8          | 13.5±1.9             | 1.3±0.2          | 23.5±2.8             | 1.5±0.4           | 59.4±10.9            |
| 0.001                   | 6          | 4.4±0.2          | 16.0±2.1             | 1.2±0.4          | 23.6±3.4             | 1.2±0.4           | 59.7±13.6            |
| Nil                     | 10         | 4.1±0.5          | 16.3±1.7             | 1.1±0.03         | 23.7±2.6             | 1.1±0.03          | 59.8±10.3            |
| 0.001 and nil combined. | 16         | 4.2±0.1          | 16.2±1.2             | 1.0±0.01         | 23.8±2.0             | 1.1±0.02          | 59.8±8.0             |
| Before vernalisation    | 11         | 1.7±0.05         | —                    | 1.4±0.05         | —                    | —                 | —                    |

the coleoptile increased in mean length from 1.7 to 4.1 mm., this difference being highly significant. Root growth, on the other hand, was negligible in the absence of sugar. This extension of the coleoptile may be due in part to incomplete imbibition of the coleoptiles measured before vernalisation; the increase is, however, too large to be attributed solely to imbibition, and indicates that some extension of the coleoptile takes place without added sugar, while root extension is entirely dependent on the external food supply.

With increasing sucrose supply the length of both coleoptiles and roots after treatment was much increased, but whereas the coleoptile length was not much affected beyond a concentration of 0.25 per cent., in the roots response occurred up to the 2 per cent. level. This may be due to the fact that coleoptile growth approaches a limiting value. Above this concentration a reduction in length of both organs occurs (Bonner, Haagensmit, and Went, 1939; Burström, 1941). From the columns of differences in Table VII it appears that no statistically significant reduction in length occurs for either coleoptile or root until the concentration falls to 0.25 per cent. This critical concentration is the same as that found for delay in flowering (Table VI).

#### 5. *Effect of changing the sucrose supply during vernalisation.*

(i) *On flowering behaviour.* In this series of experiments embryos were grown from 1 to 5 weeks either (a) on the standard medium containing sugar or (b) on a sugar-free medium. At the end of this preliminary period the embryos were transferred from one to the other medium, and kept there until a total period of 6 weeks had elapsed. Thus the embryos were exposed to a low temperature (2° C.) throughout, but some received sugar at the beginning and others at the end of the vernalisation period. Actually three concentrations of sucrose were used and the whole experiment replicated, but with the lower concentrations used (0.1, 0.01 per cent.) the results were obscured by the variability of the material, attributed to variation in stored carbohydrate content of the embryos, so that only results obtained with 2 per cent. sucrose are considered. The results on flowering are given in Table VIII.

The horizontal rows of figures marked 1 and 2 in the table give the time in days to anthesis of embryos which were transferred after the stated times. Vertically superimposed entries thus show the effect on flowering of a definite number of weeks of sugar supply given either (A) after, or (B) before a period on medium without sugar (nil medium). The dependence of the effect on the time at which sugar is supplied is shown in the row numbered 3. In all cases the standard errors are given. It appears that in every case the sugar effect is greater when applied before the starvation period, and although only one individual entry (3 weeks) reaches statistical significance the combined effect is highly significant. The row marked 4 in the table reproduces the entries in Table II; the values for 4 weeks' sucrose, given in brackets, were obtained by graphical interpolation. The entries in this row show the effects on flowering of sugar treatment *not followed* by a further low-temperature

TABLE VIII

*Effect on Flowering Behaviour (Days to Anthesis) of Transference during Vernalisation after varying periods (A) from Sugar-free Medium to 2 per cent. Sucrose; (B) from 2 per cent. Sucrose to Sugar-free Medium*

| Weeks on 2% sucrose   | 1           | 2        | 3         | 4      | 5        | 6        |
|---|-------------|----------|-----------|--------|----------|----------|
| Weeks without sucrose                                       | 5           | 4        | 3         | 2      | 1        | 0        |
| 1. A. Nil to sugar  | { 103.0±7.3 |          |           |        |          |          |
| 2. B. Sugar to nil  | { 99.0±9.1  |          |           |        |          |          |
| 3. Difference   | { 4.0±12.1  |          |           |        |          |          |
| 4. On sugar without further low temperature on nil          | >150        | >150     | 137.9±4.4 | (112)  | 86.4±2.4 | 76.7±1.3 |
| Accelerating effect of low temperature during nil period.   |             |          |           |        |          |          |
| 5. A at beginning   | { 47.0±8.6  | 52.3±6.5 | 46.2±6.1  | (24.4) | 3.2±4.0  | —        |
| 6. B at end   | { 51.0±7.5  | 68.0±2.1 | 59.3±7.3  | (37.0) | 10.8±4.1 | —        |
| 7. A per week   | { 9.4       | 13.1     | 15.4      | (12.2) | 3.2      | —        |
| 8. B per week   | { 10.2      | 17.0     | 19.8      | (18.5) | 10.8     | —        |
| Accelerating effect of sugar during low-temperature period. |             |          |           |        |          |          |
| 9. A at end   | 2.6         | 7.9      | 13.9      | 18.0   | 22.4     | } 28.9   |
| 10. B at beginning  | 6.6         | 23.6     | 27.0      | 30.6   | 30.0     |          |
| 11. A per week  | 2.6         | 3.9      | 4.6       | 4.5    | 4.5      | } 4.8    |
| 12. B per week  | 6.6         | 11.8     | 9.0       | 7.6    | 6.0      |          |



period without sugar. The differences between the values in this row and those of the first two rows give the effects of the starvation period (nil period), i.e. the effect of low temperature without added sugar supply either (A) at beginning or (B) at end of the vernalisation period. These values are given in rows 5 and 6 of the table. Since the length of the 'nil' period varies, its effect per week is presented in rows 7 and 8.

By subtraction, in the first two rows, of entries in consecutive columns from the values in the first column the effects of varying durations of 2 per cent. sucrose treatment at the beginning (A) and end (B) of the vernalisation period are found, and are presented in rows 9 and 10. Finally the effects per week of this sugar treatment are presented in rows 11 and 12.

Before stating conclusions to be drawn from entries in Table VIII the following considerations may be stressed. There are three factors which may play a part in such transference experiments: (1) absorption of sugar by the embryo, (2) immediate effect of low temperature on the embryo or on the absorbed sugar or on both, (3) after-effects on the absorbed sugar in the sense of metabolic changes initiated and maintained by low-temperature conditions.

The relative rates of processes under headings (1) and (3) are likely to be important. For if absorption of sugar is in excess of the requirements of other processes, then although immediate effects of low temperature maintained during short periods of sugar feeding may not appear later in flowering behaviour, yet large after-effects may nevertheless be induced by further low-temperature treatment owing to subsequent changes involving the absorbed sugar. If the rates of the processes under heading (3) are at first very slow, a 'lag period' in the effect of low temperature may be anticipated. In experiments involving transference from sugar to 'nil' and vice versa the order of procedure will be of importance, since if sugar is given first a storage within the embryo is possible, the result of which will become apparent later in the 'nil' period. When the 'nil' period is given first no metabolic changes directly attributable to the low-temperature effect on absorbed sugar can take place until that period is terminated. Should nevertheless an after effect of a 'nil' period applied before sugar feeding be exhibited, then other changes in the embryo as indicated under heading (2) will have to be postulated. The following conclusions from Table VIII may now be presented.

(1) The combined effect of sugar supply and low temperature, without subsequent further cold treatment, is ineffective unless prolonged beyond 2 weeks, the plants remaining completely unvernalsed (row 4 of Table VIII). Longer treatment leads to a rapid acceleration of flowering. From this it may be concluded that 2 weeks of sugar and low temperature at the beginning are ineffective, and that more than 2 weeks are required for further action (after effect of low temperature) on the absorbed sugar.

(2) The effect of the 'nil' period with ample sugar stored in the embryo is seen by comparison of entries in row 4 and row 2 of Table VIII. The results are presented graphically in Fig. 1. The differences in the ordinates of the

two curves *B* and *C* give the effect of the 'nil' period' following the period of sugar feeding; these values are given in row 6 of the table. The large effect of the 'nil' period without extra sugar supplied is taken to be due to the stored sugar or starch in the embryo. In Figs. 1 and 2 it should be noted that the ordinates represent 'degree of vernalisation', as a positive quantity measured

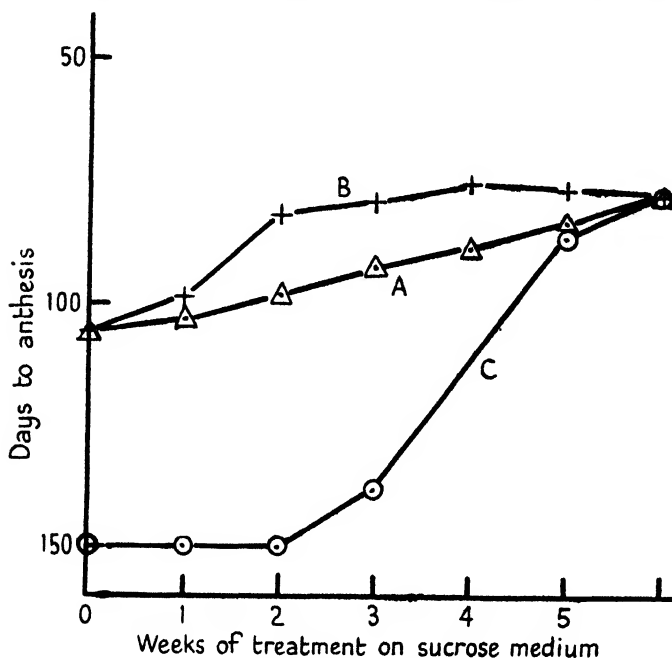


FIG. 1. Petkus winter rye. Excised embryos. Effect on flowering behaviour of supplementing the period of vernalisation on 2 per cent. sucrose medium with a period on 'nil' medium, the total duration of vernalisation being constant at 6 weeks. Curve *A*, period on 'nil' precedes that on sugar; curve *B*, 'nil' period follows that on sugar; curve *C*, no 'nil' period given. Ordinates: mean number of days to anthesis. Abscissae: duration of low temperature treatment in weeks.

by the number of days less than 150 required to reach the stage of anthesis. The maximum after-effect of sugar feeding is seen to be reached after 2 weeks' sugar treatment; at this time the sugar absorbed by the embryo has already reached a critical level and further absorption has little effect. The longer periods of sugar feeding thus act simply as 'low temperature after sugar', and since in each case the total period at low temperature is the same, the time to flowering remains constant for periods of sugar feeding greater than 2 weeks (cf. row 2, Table VIII).

(3) Fig. 1 (*C*) shows the accelerating effect on flowering of exposure to low temperature for different periods of time. Since, as has already been mentioned, after 2 weeks the critical level of sugar has been attained by the embryo, the positive effects shown in curve *C* are due solely to low temperature. Two points emerge: (*a*) there is a lag period in the low-temperature effect.

This is not due to lack of sugar as curve *B* in Fig. 1 shows; for on removal to a sugarless medium with further cold treatment the effect of the absorbed sugar becomes evident. (b) The effect of low temperature progresses with *increasing velocity* once the reaction begins. It appears probable therefore that the lag period is taken up in the elaboration by synthesis from sugar of

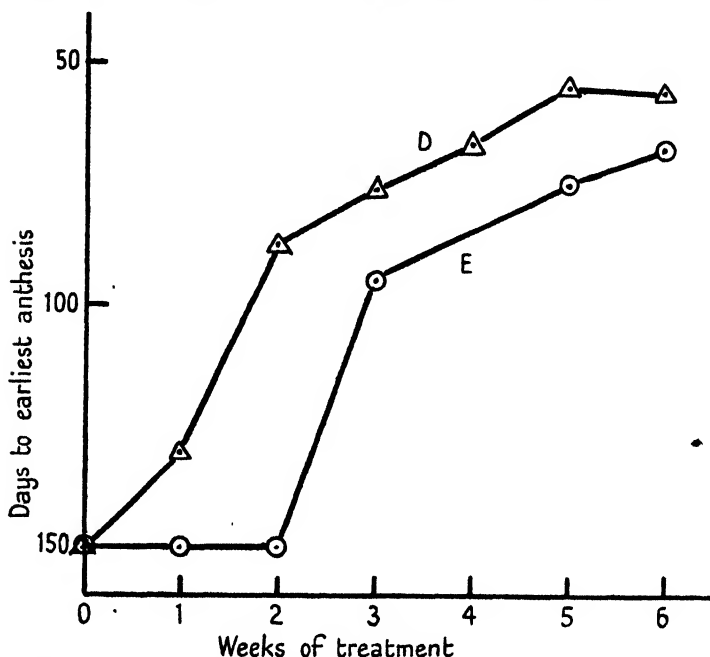


FIG. 2. Petkus winter rye. Effect of duration of vernalisation treatment on flowering of plants raised from whole grain (curve *D*) and from excised embryos vernalised on 2 per cent. sucrose (curve *E*). *Ordinates*: number of days to *earliest* anthesis in each series. *Abscissae*: duration of low-temperature treatment.

some essential component of the chain of reactions initiated by low temperature. A comparison of the effect of low temperature on the *whole grain* with the excised embryo confirms this view. As stated earlier (p. 294), the effect of varying periods of vernalisation of the whole grain was discussed in a previous paper (Purvis and Gregory, 1937), and the data there given (Table I, p. 573) are graphically shown in Fig. 2 (*D*). In this figure curve *E* relates to the same series as curve *C* in Fig. 1, but for comparison with the data available for whole grain the ordinates here give the number of days to anthesis of the *earliest* plant in each series. Here (*D*) no lag period is present, otherwise the features of the curves are similar, and indeed the curves are nearly parallel, showing that the maximum rate of the process of vernalisation by low temperature is the same in both cases. The existence of this time lag in the vernalisation of excised embryos has been confirmed in later work. The conclusion can scarcely be avoided that in the whole grain some material is supplied directly to the embryo from the endosperm or aleurone layer which

in the excised embryo has first to be synthesized, and hence the lag period. The nature of this substance is unknown; evidently, however, in so far as it is essential for vernalisation it can be synthesized by the excised embryo from cane sugar.

There remains for discussion the effect of a 'nil' period before sugar feeding (Table VIII, rows 5 and 7) and Fig. 1 (curve *A*). This aspect of the experiment is confused by the presence of stored sugar and starch in the embryo. The entries in row 5 must be read from right to left to give increasing periods without sugar. One week 'nil' period has no significant effect, but 2 weeks appear to accelerate flowering considerably; unfortunately this value is based on an interpolation. The values in row 7 of Table VIII show that the maximum effect per week is reached after 3 weeks' preliminary 'nil' period, and with increasing duration no significantly increased effect on flowering is noted (row 5). As already seen, there is a lag period of 2 weeks in the effect of low temperature on absorbed sugar (confirmed in 1941 by further experiments), so that this period of time would elapse before the stored sugar in the embryo would be effective. There appears, however, to be an additional effect of the preliminary 'nil' period so that a direct effect of low temperature on the embryo apart from its effect on sugar may be involved. The values in row 11 of Table VIII lend some support to this view, for it is here seen that after such a period the effect of added sugar on acceleration of flowering is dependent only on the duration of exposure to sugar and low temperature. This contrasts with the effect of sugar and low temperature combined acting at the beginning of germination followed by a 'nil' period. Here the rate of the induced reaction reaches a maximum at 2 weeks, which has already been identified as the lag period in the low-temperature effect. These varying effects of low temperature and sugar supplied are graphically shown in Fig. 1, curves *A* and *B*, the differences in the ordinates being the values in row 3 of Table VIII.

Without further experimental evidence the conclusions of this experiment cannot be accepted as definitive; the evidence, however, points to an effect on the embryo of low temperature apart from the direct effect of the absorbed sugar; this possibility has already been discussed in the preliminary considerations of the results shown in Table VIII.

ii. *On growth of embryos during the period of low-temperature treatment.* The data relating to growth of the coleoptiles and roots are presented in Table IX. The mode of construction of this table is similar to that employed in Table VIII. The following points may be noted. The different effects of sugar supply on the growth of the coleoptile and roots already seen in dealing with the effects of sucrose concentration are again apparent. Thus the effect on the coleoptile of sugar at low temperature (rows 11 and 12 of Table IX) is uniform throughout and not dependent upon a preceding or subsequent 'nil' period. In the root the effect of sugar is always greater when this is applied early in the period of treatment (cf. rows 11 and 12); either the preliminary starvation reduces the subsequent effect of sugar, or during early

TABLE IX

*Effect on Growth of Embryos during Vernalisation Treatment of Transference after varying Periods. (A) from Sugar-free Medium to 2% Sucrose, (B) from 2% Sucrose to Sugar-free Medium*

|  | COLEOPTILE GROWTH (mm.) |         |          |          |          |          |          |          |         |          |          |          | TOTAL ROOT GROWTH (mm.) |           |          |         |          |          |          |          |           |  |  |  |
|--|-------------------------|---------|----------|----------|----------|----------|----------|----------|---------|----------|----------|----------|-------------------------|-----------|----------|---------|----------|----------|----------|----------|-----------|--|--|--|
|  | 0                       | 1       | 2        | 3        | 4        | 5        | 6        | 0        | 1       | 2        | 3        | 4        | 5                       | 6         | 0        | 1       | 2        | 3        | 4        | 5        | 6         |  |  |  |
| Weeks on 2% sucrose                                    | 6                       | 5       | 4        | 3        | 2        | 1        | 0        | 6        | 5       | 4        | 3        | 2        | 1                       | 0         | 6        | 5       | 4        | 3        | 2        | 1        | 0         |  |  |  |
| Weeks without sucrose                                  |                         |         |          |          |          |          |          |          |         |          |          |          |                         |           |          |         |          |          |          |          |           |  |  |  |
| 1. A 'Nil', then sugar                                 | 4.2±0.12                | 6.7±0.3 | 8.1±0.6  | 11.9±0.9 | 15.2±0.7 | 14.1±0.7 | 20.4±1.9 | 1.2±0.04 | 2.7±0.4 | 6.9±1.3  | 18.2±3.0 | 21.1±2.8 | 45.3±4.8                | 60.9±12.5 | 1.2±0.04 | 2.7±0.4 | 6.9±1.3  | 18.2±3.0 | 21.1±2.8 | 45.3±4.8 | 60.9±12.5 |  |  |  |
| 2. B Sugar, then 'Nil'                                 | —                       | 6.5±0.6 | 12.3±0.5 | 12.0±0.9 | 16.0±0.6 | 15.9±0.5 | —        | —        | 4.2±0.8 | 13.6±2.3 | 24.2±3.3 | 55.0±7.6 | 65.0±3.3                | —         | —        | 4.2±0.8 | 13.6±2.3 | 24.2±3.3 | 55.0±7.6 | 65.0±3.3 | —         |  |  |  |
| 3. Effect of sugar priority                            | —                       | —       | 0.2±0.6  | 4.2±0.9  | 0.1±1.5  | 0.8±1.2  | 1.8±1.0  | —        | 1.5±0.8 | 6.7±2.2  | 6.0±4.5  | 33.9±6.5 | 19.7±6.9                | —         | —        | 1.5±0.8 | 6.7±2.2  | 6.0±4.5  | 33.9±6.5 | 19.7±6.9 | —         |  |  |  |
| 4. On sugar only, no further low-temperature treatment | 1.7±0.10                | 5.5±0.2 | 6.3±0.5  | 9.4±0.5  | (12.0)   | 14.4±1.3 | 20.4±1.9 | 1.4±0.1  | 3.0±0.7 | 6.6±1.6  | 19.8±2.2 | (33.0)   | 47.7±3.4                | 60.9±12.5 | 1.4±0.1  | 3.0±0.7 | 6.6±1.6  | 19.8±2.2 | (33.0)   | 47.7±3.4 | 60.9±12.5 |  |  |  |
| Effect of 'Nil' period.                                |                         |         |          |          |          |          |          |          |         |          |          |          |                         |           |          |         |          |          |          |          |           |  |  |  |
| 5. A at beginning                                      | 2.5                     | 1.2     | 1.8      | 2.5      | (3.2)    | —        | —        | —        | —       | 0.3      | —        | (11.0)   | —                       | —         | —        | —       | 0.3      | —        | —        | —        | —         |  |  |  |
| 6. B at end  | —                       | 1.0     | 6.0      | 2.6      | (4.0)    | 1.5      | —        | —        | 1.2     | 7.0      | 4.4      | 22.0     | 17.3                    | —         | —        | 1.2     | 7.0      | 4.4      | 22.0     | 17.3     | —         |  |  |  |
| 7. A per week  | 0.40                    | 0.24    | 0.45     | 0.83     | (1.6)    | —        | —        | —        | —       | 0.06     | —        | —        | —                       | —         | —        | —       | 0.06     | —        | —        | —        | —         |  |  |  |
| 8. B per week  | —                       | 0.20    | 1.5      | 0.86     | (2.0)    | 1.5      | —        | —        | 0.2     | 1.8      | 1.5      | 11.0     | 17.3                    | —         | —        | 0.2     | 1.8      | 1.5      | 11.0     | 17.3     | —         |  |  |  |
| Effect of sugar during low-temperature period.         |                         |         |          |          |          |          |          |          |         |          |          |          |                         |           |          |         |          |          |          |          |           |  |  |  |
| 9. A at end  | —                       | 2.5     | 3.9      | 7.7      | 11.0     | 9.9      | 16.2     | —        | 1.5     | 5.7      | 17.0     | 19.9     | 44.1                    | 59.7      | —        | 1.5     | 5.7      | 17.0     | 19.9     | 44.1     | 59.7      |  |  |  |
| 10. B at beginning                                     | —                       | 2.3     | 8.1      | 7.8      | 11.8     | 11.7     | —        | —        | 3.0     | 12.4     | 23.0     | 53.8     | 63.8                    | —         | —        | 3.0     | 12.4     | 23.0     | 53.8     | 63.8     | —         |  |  |  |
| 11. A per week   | —                       | 2.5     | 1.9      | 2.6      | 2.7      | 2.0      | 2.7      | —        | 1.5     | 2.8      | 5.7      | 4.9      | 8.8                     | 9.9       | —        | 1.5     | 2.8      | 5.7      | 4.9      | 8.8      | 9.9       |  |  |  |
| 12. B per week   | —                       | 2.3     | 4.0      | 2.6      | 2.9      | 2.3      | —        | —        | 3.0     | 6.2      | 7.7      | 13.4     | 12.8                    | —         | —        | 3.0     | 6.2      | 7.7      | 13.4     | 12.8     | —         |  |  |  |

sugar treatment sugar is absorbed in excess of the immediate requirements of the embryo. In this respect flowering behaviour more closely follows root growth than that of the coleoptiles.

The root growth as measured weekly increases with the period of application of the sugar treatment, indicating an autocatalytic effect on root growth. This is confirmed by the data in row 4 of the table, where during the first 5 weeks the length of the root is seen to double each week.

The effect on the coleoptile of the 'nil' period (rows 7 and 8) is noticeable, even when applied *before* sugar; whereas in the root a preliminary 'nil' period is without effect or is detrimental. After sugar has been supplied (row 8) a marked effect on root growth of the 'nil' period is noted, but only when the preliminary sugar feeding is protracted. In the coleoptile even short preliminary sugar treatment is effective. The differential reaction of coleoptile and root is clearly related to the effective sugar level required for maximum growth. This for the coleoptile is supplied by a concentration of 0.25 per cent. sucrose, whereas for roots 2 per cent. is required. Unless therefore the preliminary period is of long duration, making possible a storage of sugar, the subsequent 'nil' period is without effect on the root.

#### 6. *Effect of the chemical nature of the carbohydrate supplied.*

i. *On flowering behaviour.* A variety of carbohydrates as well as alcohols and organic acids were tested. In each case the substance was added to the culture medium on which the excised embryos were grown during vernalisation at a molecular concentration equivalent to that of 2 per cent. sucrose, which was used at the standard. The effects of these substances on vernalisation are given in Table X, in which the number of days to anthesis is given for each medium, with the replication of each series. The third column shows the acceleration in flowering due to the presence of each substance compared with the control series without sugar and the fourth shows the significance of these effects. It will be remembered that, in the absence of any carbohydrate supply, some degree of vernalisation was effected, since this control series flowered in 106 days (see Table VI, p. 296). The fifth column gives the concentration of sucrose approximately equal in effect to the substance in question (see Table VI), while the sixth shows the order of flowering of the variously treated series. Finally the mean tillering of each series is given in order to show that lateness of flowering after vernalisation on certain media is not due simply to weakened growth, since the tillering is increased in the same way as in unvernalsed plants of winter rye.

From columns 3 and 4 it appears that pyruvic acid and mannose significantly retarded flowering, while a small retardation followed the use of mannitol. The tillering behaviour tends to confirm an actual retarding effect. Arabinose and fructosan approximated the control medium in value. Maltose, galactose, raffinose, and glycerol were all equivalent to 0.01 per cent. sucrose; their accelerating effects, though considerable (10–17 days), were not statistically

TABLE X

*Effect of the chemical Composition of the Carbon Supply during Low-temperature Treatment on the flowering Behaviour of excised Embryos*

|                       | Replicates.  | Days to anthesis. | Difference from control without sugar. | Significance P. | Equiv. % of sucrose. | Order of flowering | Tillers per plant. 102 days. |        |        |
|-----------------------|--------------|-------------------|--|-----------------|----------------------|--------------------|------------------------------|--------|--------|
|                       |              |                   |  |                 |                      |                    | Shooting.                    | Green. | Total. |
| Ribose .              | .            | .                 |  |                 |                      |                    |                              |        |        |
| Arabinose             | 7            | 79.7 ± 2.0        | 25.9 ± 10.2                            | 0.02            | 1.0                  | 3                  | 6.6                          | 1.3    | 7.9    |
| Xylose .              | 4            | 108.5 ± 2.7       | 2.9 ± 14.7                             | —               | nil                  | 12                 | 3.3                          | 9.8    | 13.1   |
| Glucose               | 6            | 84.2 ± 2.5        | 21.4 ± 11.0                            | 0.05            | 0.25                 | 5                  | 3.6                          | 3.0    | 6.6    |
| Mannose               | 8            | 80.4 ± 5.7        | 25.2 ± 9.7                             | 0.02            | 1.0                  | 4                  | 4.9                          | 2.7    | 7.6    |
| Galactose             | 3            | 142.0 ± 0.0       | -36.4 ± 15.2                           | 0.05            | —                    | 15                 | 0.0                          | 32.0   | 32.0   |
| Fructose              | 6            | 96.5 ± 7.8        | 9.1 ± 11.8                             | —               | 0.01                 | 9                  | 3.0                          | 1.7    | 4.7    |
| Sucrose               | 8            | 74.2 ± 1.4        | 31.3 ± 9.5                             | 0.01            | 2.0                  | 1                  | 4.1                          | 1.8    | 5.9    |
| Maltose               | 46           | 76.7 ± 1.1        | 28.9 ± 4.3                             | 0.01            | —                    | 2                  | 6.3                          | 7.0    | 13.3   |
| Raffinose             | 5            | 94.0 ± 13.0       | 11.6 ± 13.8                            | —               | 0.01                 | 8                  | 4.4                          | 6.3    | 10.7   |
| Fructosan*            | 6            | 92.3 ± 10.3       | 13.2 ± 12.5                            | —               | 0.01                 | 7                  | 3.1                          | 10.0   | 13.1   |
| Glycerol              | 5            | 108.2 ± 14.2      | -2.6 ± 14.1                            | —               | nil                  | 11                 | 1.5                          | 14.0   | 15.5   |
| Mannitol              | 7            | 88.8 ± 7.5        | 17.0 ± 11.2                            | —               | 0.01-0.1             | 6                  | 3.8                          | 4.6    | 8.4    |
| Pyruvic acid          | 7            | 115.4 ± 9.8       | -9.9 ± 11.9                            | —               | nil                  | 13                 | 2.4                          | 10.0   | 12.4   |
| Malic acid            | 3            | 138.7 ± 10.6      | -33.1 ± 15.2                           | 0.05            | nil                  | 14                 | 0.0                          | 38.7   | 38.7   |
| Succinic acid.        | No survivors |                   |  |                 |                      |                    |                              |        |        |
| Control without sugar | 16           | 105.6 ± 6.6       | —                                      | —               | —                    | 10                 | 4.3                          | 8.8    | 13.1   |

\* Concentration based on molecular weight of fructose. A second series with 10 times this concentration was toxic.

significant. When the high variability of the material is remembered it will be apparent that these substances cannot be dismissed as without value in the vernalisation process. Ribose, glucose, fructose, and xylose gave significant accelerations, the last barely so; of these fructose alone equalled sucrose in efficiency.

The fact which emerges from these data is the apparent lack of relationship between the structure of the molecules concerned and their effect. Two of the pentoses were effective while the third was quite inert. The three aldohexoses again differed in behaviour and were inferior to fructose. Of the more complex saccharides sucrose alone was significantly effective. The fructose molecule is of high value either alone or in sucrose, but fructosan at the concentration in Table X was without effect.

TABLE XI

*Effect of the Chemical Composition of the Carbon Supply on the Growth of excised Embryos during Low-temperature Treatment*

|                       | COLEOPTILES                   |  |                 |                               | TOTAL ROOTS                   |  |                 |                               |
|-----------------------|-------------------------------|--|-----------------|-------------------------------|-------------------------------|--|-----------------|-------------------------------|
|                       | Length after 6 weeks at 1° C. | Difference from control without sugar. | Significance P. | Equiv. concn. (%) of sucrose. | Length after 6 weeks at 1° C. | Difference from control without sugar. | Significance P. | Equiv. concn. (%) of sucrose. |
| Ribose                | 9.6 ± 0.9                     | 5.4 ± 0.6                              | 0.01            | 0.01                          | 6.2 ± 1.6                     | 5.1 ± 1.0                              | 0.01            | 0.1                           |
| Arabinose             | 4.5 ± 0.3                     | 0.3 ± 0.3                              | —               | nil                           | 1.3 ± 0.3                     | 0.2 ± 0.4                              | —               | nil                           |
| Xylose                | 12.8 ± 1.2                    | 8.6 ± 0.7                              | 0.01            | 0.01-0.1                      | 8.0 ± 2.0                     | 6.9 ± 1.2                              | 0.01            | 0.25                          |
| Glucose               | 9.6 ± 0.7                     | 5.4 ± 0.5                              | 0.01            | 0.01                          | 43.4 ± 2.7                    | 42.3 ± 1.9                             | 0.01            | 1.0                           |
| Mannose               | 3.8 ± 0.2                     | -0.4 ± 0.3                             | —               | nil                           | 1.2 ± 0.4                     | 0.1 ± 0.2                              | —               | nil                           |
| Galactose             | 7.3 ± 0.7                     | 3.1 ± 0.5                              | 0.01            | 0.01                          | 2.7 ± 0.8                     | 1.6 ± 0.6                              | 0.02            | 0.01                          |
| Fructose              | 12.1 ± 0.7                    | 7.9 ± 0.5                              | 0.01            | 0.01-0.1                      | 36.9 ± 4.5                    | 35.8 ± 3.1                             | 0.01            | 0.5-1.0                       |
| Sucrose               | 20.4 ± 1.9                    | 16.2 ± 1.2                             | 0.01            | —                             | 60.9 ± 12.5                   | 59.8 ± 8.0                             | 0.01            | —                             |
| Maltose               | 11.5 ± 1.3                    | 7.3 ± 1.2                              | 0.01            | 0.01-0.1                      | 27.3 ± 6.5                    | 26.2 ± 3.4                             | 0.01            | 0.5                           |
| Raffinose             | 11.6 ± 1.5                    | 7.4 ± 0.9                              | 0.01            | 0.01-0.1                      | 25.4 ± 6.5                    | 24.3 ± 3.8                             | 0.01            | 0.5                           |
| Fructosan             | 4.4 ± 0.2                     | 0.2 ± 0.2                              | —               | nil                           | 1.4 ± 0.3                     | 0.3 ± 0.3                              | —               | nil                           |
| Glycerol              | 8.9 ± 1.2                     | 4.7 ± 0.8                              | 0.01            | 0.01                          | 2.4 ± 0.5                     | 1.3 ± 0.4                              | 0.01            | 0.01                          |
| Mannitol              | 4.4 ± 0.3                     | 0.2 ± 0.3                              | —               | nil                           | 1.1 ± 0.3                     | 0.1 ± 0.4                              | —               | nil                           |
| Pyruvic acid          | 4.2 ± 0.2                     | —                                      | —               | nil                           | 1.6 ± 0.2                     | 0.5 ± 0.3                              | —               | nil                           |
| Control without sugar | 4.2 ± 0.1                     | —                                      | —               | —                             | 1.1 ± 0.02                    | —                                      | —               | —                             |

ii. *On growth of the embryos during vernalisation treatment.* The growth of the embryos on the various media at room temperature was not systematically studied, but growth during treatment was examined in some detail, and the results are set out in Table XI. This table deals with measurements of coleoptiles and roots of those embryos only which survived till the end of the experiment; the replication is therefore the same as in Table X.

As stated on page 298, the length of the embryonic coleoptile is more than doubled during 6 weeks on the medium without sugar, while in the same conditions the roots make no growth. This applies also to the media with arabinose, fructosan, mannitol, mannose, and pyruvic acid, which were either inert in vernalisation or even retarded flowering. In all these, a significant coleoptile extension, unaccompanied by root growth, occurred during vernalisation treatment. It may, then, be inferred that these media are not detrimental to the embryos, but cannot be utilized by them.

For growth, sucrose is the most effective sugar tried. The remaining eight



substances significantly increased the growth of both coleoptiles and roots, but to varying degrees. With all of them coleoptile growth was approximately equal to that on 0.01–0.1 per cent. sucrose and significantly less than on the standard 2 per cent. medium. Though these eight substances are close together in relation to sucrose concentrations they differ significantly from each other. Thus coleoptile growth on galactose is exceeded by that on glucose by  $2.3 \pm 1.0$  mm. and by that on fructose by  $4.8 \pm 1.0$  mm. In considering these results it must be remembered that the concentrations used are molecular equivalents of 2 per cent. sucrose; hexoses were thus added at 1 per cent. concentration and the pentoses at a slightly lower strength. Raffinose alone was at a higher concentration than sucrose. When this is taken into consideration it still appears that coleoptile extension is effected by a lower concentration of sucrose than of any other sugar tested. The growth response of root was in general somewhat different from that of coleoptiles. The mean values covered a wider range as a result of the autocatalytic nature of the process and, in the main, was equivalent to much higher levels of sucrose concentration. Further, the relative value of the different substances for root and coleoptile growth was not the same; notably, ribose and xylose were inactive in root production.

Comparing Tables X and XI several points of interest appear. Maltose and raffinose have strikingly similar effects both on flowering behaviour and on growth. The unexpected efficiency of the pentoses, ribose and xylose, in vernalisation is reflected in their effect on coleoptile growth, but here xylose gives the higher value. Root growth is poor on both these media, but even so the total root length on ribose exceeds that on arabinose by  $4.9 \pm 2.1$  mm. Glucose, while equalling ribose for flowering and coleoptile growth, produces eight times as much root. No relationship between the constitution of the various sugars and their effects can be recognized. The availability of glycerol for both vernalisation and growth is noteworthy. The relatively high value for growth of sucrose compared with glucose and fructose may be due to the presence in its molecule of the furanose form of fructose, in accordance with the hypothesis of Dragone-Testi (1935). If this is so, it would appear that the pyranose form is no less effective for vernalisation than the more active form.

#### *7. The relation of the degree of vernalisation to the growth of the embryo.*

A consideration of the data presented in this paper reveals beyond doubt that a high degree of vernalisation is, in general, associated with considerable embryo growth (compare Tables V and VI, VIII and IX). Lojkin (1936) records an increase in the response of winter wheat to vernalisation when an increased moisture level permitted more embryo growth during treatment. The question thus arises if the function of the sugar in the vernalisation process is simply to provide the necessary amount of growth. Sen and Chakravarti (1942) have shown that in mustard vernalisation is possible in imbibed but unchitted seed, though it proceeds more slowly when the seed

coat is unbroken. In such seed growth during treatment must be very slight. Similarly in the work recorded here embryos vernalised on sugar-free medium can respond to treatment, while a minimal amount of growth is in progress. Among embryos thus treated no more extensive growth is associated with

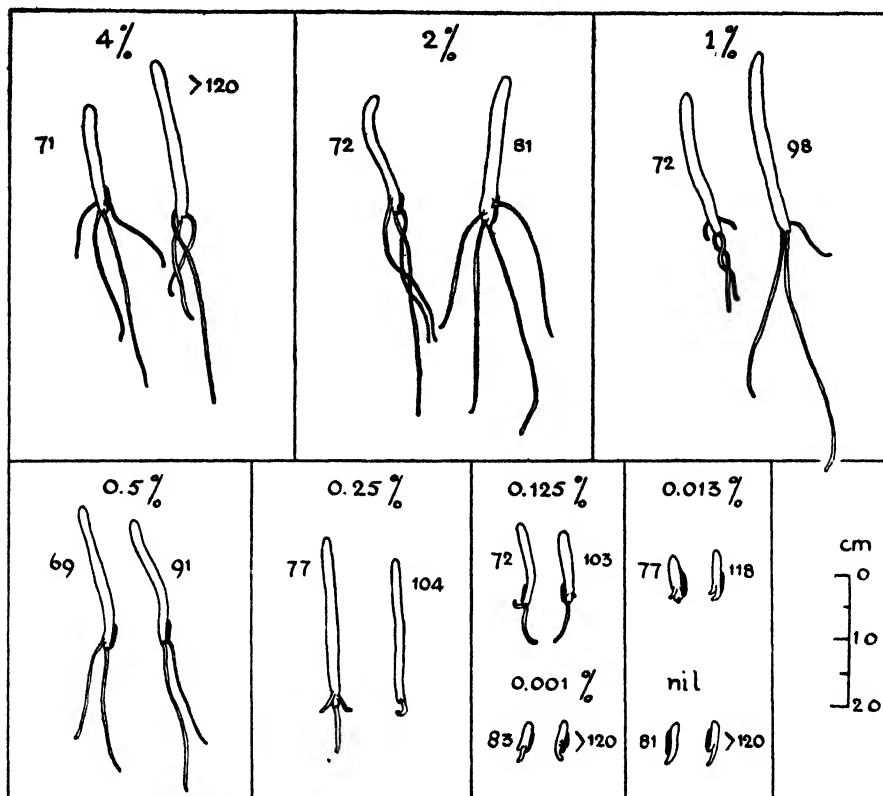


FIG. 3. Outlines of excised embryos after 6 weeks of low-temperature treatment on media containing sucrose of concentrations given. The embryos shown are those which flowered earliest and latest in each series, each with the number of days from planting to flowering.

large vernalisation response than in its absence. This is further shown in the series vernalised with carbohydrate present; within each series with a particular sugar supply, the growth of the individual embryos is quite unrelated to their varied response to vernalisation. In Figs. 3 and 4 the outlines of the 'earliest' and 'latest' embryos from each variant are shown. In many series the 'earliest' embryo is smaller than the 'latest' and, moreover, some embryos on low sugar media and of little growth are earlier than much larger but retarded individuals vernalised in the presence of more sugar. Correlation coefficients have been obtained in later experiments with higher replication than those described above. In these the correlation between growth and days to anthesis is low and not significant. In only one series was the correlation coefficient higher than 0.2 and in that case it failed in the

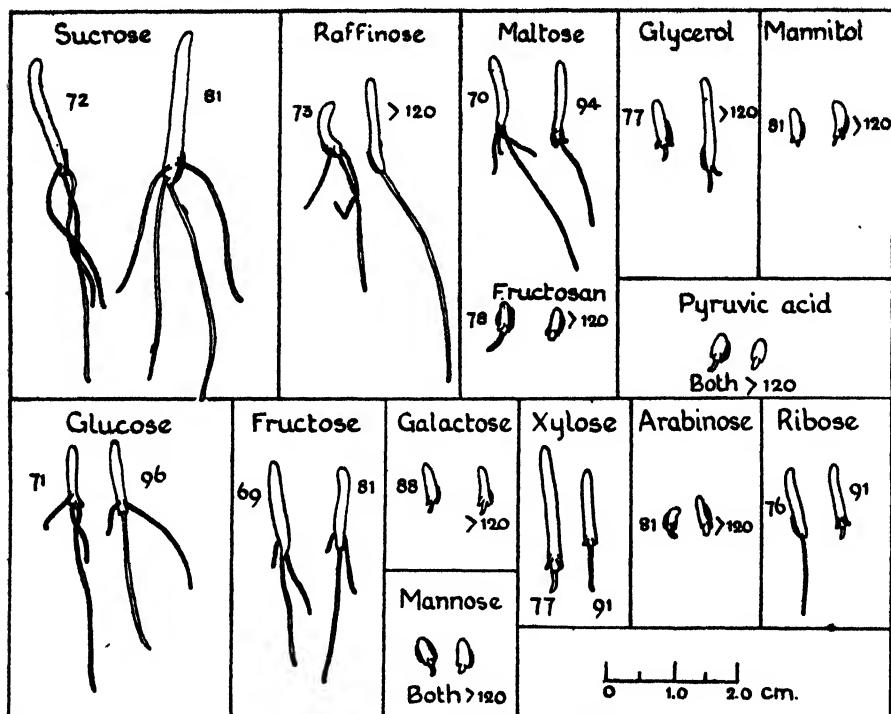


FIG. 4. Outlines of excised embryos after 6 weeks of low-temperature treatment on media containing a range of substances at concentrations equivalent to 2 per cent. sucrose. The embryos shown are those which flowered earliest and latest in each series, each with the number of days from planting to flowering.

TABLE XII

*Coefficients of Correlation between Days to Anthesis and Growth of Embryos during Vernalisation*

|                              | <i>r</i> | <i>n</i> | Significant value of <i>r</i><br>for <i>P</i> = 0.05 |
|------------------------------|----------|----------|--|
| Coleoptile lengths . . . . . | -0.17    | 58       | 0.2585   |
|                              | -0.16    | 36       | 0.3291   |
|                              | -0.16    | 18       | 0.4683   |
| Total root lengths . . . . . | -0.44    | 18       | 0.4683   |
|                              | +0.03    | 34       | 0.3388   |
|                              | +0.18    | 33       | 0.3440   |

test for significance. It should be noted that a negative correlation between 'days to anthesis' and embryo length would indicate a positive relationship between the degree of vernalisation and growth. In several other series the absence of correlation was sufficiently indicated by scatter diagrams. Again, the abnormally low embryo growth in 1940 was not associated with reduced flowering response to low-temperature treatment. Further evidence that vernalisation and growth vary independently with the sugar supply is found

in the fact established above, that the two processes respond somewhat differently to changes in the chemical composition of the carbohydrate supply.

Despite the undoubted correspondence between growth and vernalisation, these facts indicate that there is a parallel dependence of the two processes on sugar, rather than an indirect control of vernalisation by sugar through its effect on growth.

#### DISCUSSION

Vernalisation of excised embryos is independent of the nitrogen supplied in the medium on which they are grown during vernalisation. Both the concentration and the form in which nitrogen is presented are without effect on the flowering behaviour of the plants. It would appear, then, either that nitrogen plays no part in the vernalisation process, or that the supply already in the dormant embryo is adequate. Growth also is little changed in the absence of nitrates, though somewhat increased when nitrates are replaced by suitable organic compounds. Since nitrogen is essential to growth, it may be supposed that the embryo contains a reserve sufficient for considerable growth in the absence of nitrate in the medium (Table IV). This reserve is conceivably adequate for any part of the vernalisation process that may require nitrogen; the experiment thus does not rule out the possibility that nitrogen enters into the reactions concerned.

The presence of sugar in the medium appears to be necessary for complete vernalisation, though certain individual embryos can be partially vernalised without any external source of sugar. Thus the presence of sucrose in the medium substantially accelerates the process, and to a degree increasing with the concentration in which it is supplied (Table V). Hence, again two conclusions are possible: either the embryo contains in itself a source of sugar which serves for partial vernalisation, or the vernalisation process is not dependent on the presence of sucrose but is accelerated by it. The great variability of the embryos after vernalisation on low sugar concentrations and the reduction of this variability when sugar is no longer in short supply (see standard errors in Table V) suggest that the partial vernalisation without added sugar is due to utilisation of limited supplies in the embryo. It has already been pointed out that embryos contain carbohydrate, and the considerable growth which occurs at room temperature without sugar confirms this. This 'residual growth' reaches a maximum in 4 to 5 days at 20° C. and then ceases. At 1° C. residual growth also continues for a time and then stops, but at a much lower level than at 20° C. though this small increment is always statistically significant. Clearly, as growth in both cases reaches a limit, the difference is not due to the differential temperature effect on growth rate. This effect of low temperature in limiting the total growth possible, as distinct from the growth rate, was further demonstrated by direct experiment. In 1940 the 6 weeks' period of vernalisation on 'nil' medium, was preceded in one series by 4 days on the same medium at 20° C. In a parallel series the period at the higher temperature *followed* vernalisation.

Thus the aggregate treatment in the two series was identical, the difference being only in the order in which the two temperatures were applied. This experiment was repeated in 1941, when the vernalisation period was extended to 8 weeks. The growth of the embryos is given in Table XIII. In 1941 the embryos were not planted; thus no flowering data are available.

TABLE XIII

*Growth of Embryos on sugarless Medium with Period at high Temperature (A) preceding and (B) following Vernalisation*

| Treatment.  | Coleoptile (mm.). |           | Total roots (mm.). |           | Days to Anthesis.<br>1940 |
|---|-------------------|-----------|--------------------|-----------|---------------------------|
|   | 1940              | 1941      | 1940               | 1941      |                           |
| A. Four days starvation at 20° C. before vernalisation on 'nil' medium. | 5.3 ± 0.3         | 8.1 ± 0.9 | 6.6 ± 0.9          | 6.9 ± 1.3 | > 145                     |
| Replicates  | 6                 | 6         | 6                  | 6         | 6                         |
| B. Four days starvation at 20° C. after vernalisation on 'nil' medium.  | 3.5 ± 0.3         | 4.8 ± 0.6 | 3.4 ± 0.2          | 3.5 ± 0.3 | 98.5                      |
| Replicates  | 6                 | 7         | 6                  | 7         | 6                         |
| Difference  | 1.8 ± 0.4         | 3.3 ± 1.3 | 3.2 ± 0.9          | 3.4 ± 1.5 | —                         |

In Table VII it was shown that after 6 weeks' vernalisation the mean length of the coleoptile was 4.1 mm. and that of the roots 1.1 mm. Thus, although growth had ceased at low temperature some further growth of roots was possible when the temperature was raised. This further growth was, however, much reduced by the previous low-temperature treatment, so that the total growth was significantly less than that which resulted when the high-temperature period preceded vernalisation. This suggests, though it does not prove, that part of the limited sugar supply of the embryo is used up in the course of vernalisation, taking part in the reactions concerned. This question will receive further discussion in the light of later experiments.

#### SUMMARY

A method of culturing isolated embryos of winter rye in a sterile condition on an agar medium is described.

By this method the part played by nitrogen and by sugars in the process of vernalisation has been studied.

Throughout the work the excision of the embryos was facilitated by partial imbibition of the grain. It is shown, however, that when this preliminary soaking is omitted, vernalisation still remains possible. Thus parts of the grain other than the embryo are not essential to the process, though they may contribute to it.

No added nitrogen is needed for either vernalisation or growth of the embryos during treatment.

When carbohydrate is omitted, vernalisation of excised embryos is partially successful. This is attributed to residual carbohydrates in the embryo tissue. With rising concentration of sucrose in the medium the acceleration of flowering is increased and is maximal with 2 per cent. sugar. Growth of the embryos during low-temperature treatment is similarly conditioned by the sugar concentration. The general conclusion is reached that sugar takes part in the reactions involved in vernalisation.

Excised embryos differ from whole grain in the extension of the minimal duration of treatment required for any acceleration of flowering. For excised embryos at least 3 weeks' treatment is required, while whole grain sown in early May shows some response to durations of less than 1 week. It is suggested that during this period some substance is synthesized which is essential to vernalisation. In whole grain this is supplied to the embryo by the endosperm or aleurone layer.

Transference experiments in which sugar was present in the medium during part only of the 6 weeks' cold treatment show that the sugar is more effective if supplied early. It is absorbed more rapidly than it is used and in rather more than 2 weeks reaches a critical level in the embryo, after which its continued supply is without effect even though the low-temperature treatment is continued. There is also evidence of a direct effect of low temperature on the embryo apart from reactions in which sugar is involved.

The relative value of other carbohydrates, used to replace sucrose in the culture medium, varies greatly, and its relationship with their chemical constitution remains obscure.

Though embryo growth and degree of vernalisation depend on the concentration of sucrose it is considered that both vary with the sucrose supply but otherwise are independent of each other.

In conclusion the author wishes to thank Professor F. G. Gregory for his suggestions and criticism in the course of this work. In 1939 the work was carried out at Chelsea Physic Garden and in 1940 was transferred to East Malling Research Station. The vernalisation treatments after 1939 were carried out at the Low Temperature Laboratory, Ditton. To the Curator of the Chelsea Physic Garden, to Dr. R. G. Hatton and to Dr. C. West the author tenders her thanks for the facilities placed at her disposal with such readiness.

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# On a Blue-green Cryptomonad, *Chroomonas Nordstedtii* Hansg.

BY

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With six Figures in the Text

IN view of our scanty knowledge of Cryptomonads and the absence of a monograph, there are serious difficulties in the identification of any but the commonest forms encountered. The following notes and drawings, although not complete, contribute some points towards a better understanding of the blue-green Cryptomonads.

In working out the population of cultures of diverse mud samples from the bays of Windermere considerable numbers of a flagellate were found. The two best samples came from stations far apart, viz. (1) from the *Littorella* sward at a depth of 1.5 m. on the east shore of the lake, near the mouth of Troutbeck; (2) from Congo bay at a depth of 3 m. on the west shore, near Low Wray. Station 1 is a stony shore with a dominant vegetation of *Littorella uniflora*, while station 2 is a muddy bay, rich in organic substances with abundant rooted vegetation and epiphytes (Godward, 1937). The two localities belong to almost opposite extremes and ecologically are essentially different so that no conclusions as to the ecological requirements of the form in question can be drawn; it would appear to be distributed all over the lake. Sample 1 was collected in January, sample 2 in October. In both cases a nutritive solution, Benecke N, was added as the samples were part of a large-scale experiment on mud samples kept under a variety of different conditions.

The cultures were kept near a north window at room temperature, and a few months later, after a succession of algal communities had appeared, a bright blue-green film, consisting exclusively of a Cryptomonad, was visible to the naked eye floating on the surface, attached to the sides of the glass vessel. A number of Chlorophyceae, Diatoms, Chrysophyceae, and other Cryptomonads were present in addition to the blue-green unicellular organism. Most of the cells of the latter were in a non-motile stage, forming a closely packed palmella stage, for the greater part only one layer in thickness. There was a marked contrast of colours in the cells. The chromatophore was a brilliant blue with a slightly greenish tint, similar in colour to *Asterocytis*, while the centre of the cell contained bright red pigment granules of the colour of carmine, this unusual shade being possibly in part determined by the contrast afforded by the brilliant blue.



A culture of these cells was made on nutritive agar where they developed well, so that various stages could be more closely studied. Fig. 1 shows cells from the surface film. These are packed with globular grains of Cryptomonad starch of various sizes, obscuring the shape and outline of the chromatophore as well as in some cases the pyrenoid. One or two carmine red pigment

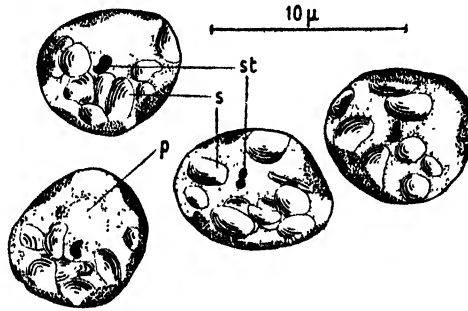


FIG. 1. *Chroomonas Nordstedtii* Hansg. Non-motile cells from surface film of culture solution; *p*, pyrenoid; *s*, starch or leucosin; *st*, stigma.

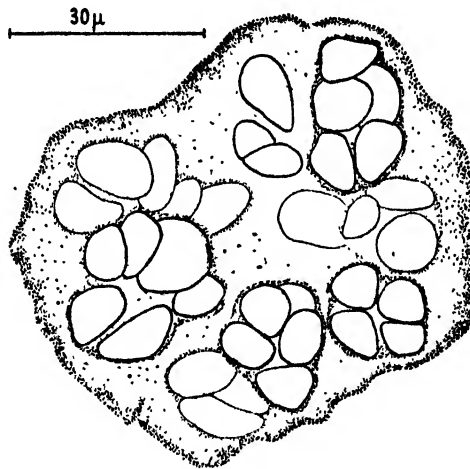


FIG. 2. *Chroomonas Nordstedtii* Hansg. Palmella stage developed on nutrient agar. Several mucilaginous sheaths can be seen.

granules, the eyespots, are seen in the centre. These cells have not undergone division for some time, as shown by the accumulation of reserve material, although those with two eyespots seem to have passed through the early stages of a division, which was then inhibited.

Fig. 2 is a palmella, developed on agar, in which frequent divisions take place. Only occasional motile cells are observed, usually along the edge of the palmella. Here as in other cultures of flagellates, the motile form appears only where there is a sufficiently thick layer of condensed moisture on the agar surface. The flagellate stage often also develops readily from the palmella

if a drop of nutritive solution is added. Such active cells show the structure clearly, as most of the starch and other granules have disappeared.

Fig. 3 shows a typical cell viewed from the ventral side. The shape is irregularly ovoid, though especially in the non-motile condition somewhat variable. There is one large parietal chromatophore which is always a transparent, slightly greenish, blue colour. It is deeply notched at the anterior and completely fills the cell at the posterior end. Occasionally, however, there is a small notch in the chromatophore at the latter end which is occupied by a highly refractive body in question, which is nearly always labelled 'nucleus', but I am of the opinion that this is a misinterpretation. Closely apposed to the chromatophore is a very distinct pyrenoid of large size and usually rounded, though sometimes slightly angular, in shape. This is usually covered by two starch envelopes.

Immediately adjoining the pyrenoid, and sometimes seemingly attached to it, is the carmine red stigma which is always found in the centre or even in the posterior half of the cell. In many cells two distinct pigment granules can be seen side by side. This probably indicates an early stage of cell division which may or may not be completed; in the latter case this results in vegetative cells with a double eyespot. Closely apposed to the chromatophore are starch grains of various sizes which in non-dividing cells may become very large (Fig. 1). In the anterior half of the cell the trichocysts probably mark the position of the furrow, although this was never clearly observed. The number of small highly refractive trichocysts varies between 4 and 5 on each side. A distinct contractile vacuole lies near the periphery of the cell. At about 15° C. the interval between two contractions varies between 10 and 14 seconds.

The motile cells show a more constant shape with a truncate anterior and a rounded posterior end (Fig. 4). Two unequal flagella are inserted in a slightly ventral position. They invariably show different movements, one remaining straight while the other lashes quickly to and fro. The difference is best seen on the agar surface when the liquid film is extremely thin and does not allow of rapid movement. It was impossible to determine whether the different behaviour is linked with the relative lengths of the flagella or a difference in structure. The one is usually about two-thirds of the length of the cell, the other slightly shorter. The size, as well as the relative proportions of the cells, varies considerably (Fig. 4a, b, and Fig. 2). The range is much the same for both motile and non-motile cells, 7-9.8  $\mu \times$  4.2-6.3  $\mu$  in the

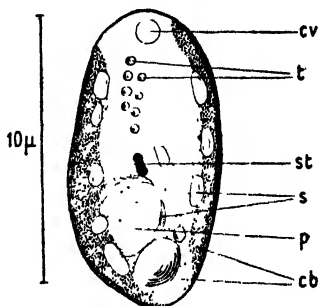
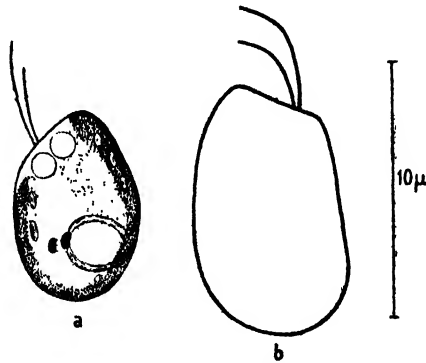


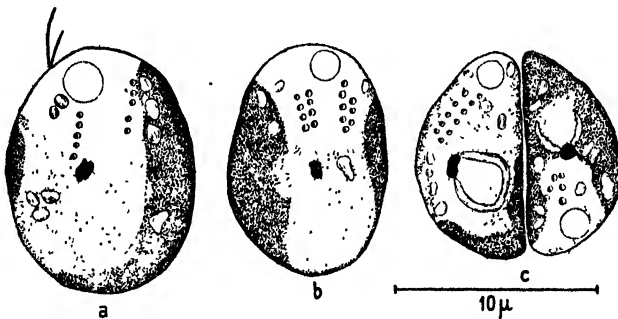
FIG. 3. *Chroomonas Nordstedtii* Hansg. Typical structure of an active, non-motile cell; *cb*, chromatophore; *cv*, contractile vacuole; *p*, pyrenoid; *s*, starch; *st*, stigma; *t*, trichocysts.

non-motile and  $7\text{--}9.8\ \mu \times 5\text{--}7\ \mu$  in the motile stage. As in many other flagellates, any non-motile cell may apparently become motile if conditions are suitable.

Division takes place in the motile or non-motile stage (Figs. 4*a* and 5*a-c*). The sequence of division of the various cell-organs may alter from individual



FIGS. 4*a* and *b*. *Chroomonas Nordstedtii* Hansg. Motile cells; 4*a*. Early stage of division, chromatophore dividing, contractile vacuole and stigma divided. 4*b*. Outline to show size of large cell.



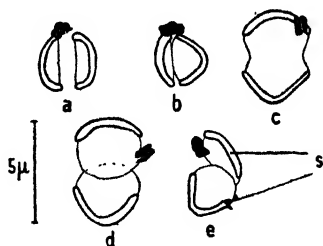
FIGS. 5*a-c*. *Chroomonas Nordstedtii* Hansg. Stages of cell division; in 5*a* one pair of daughter flagella developed.

to individual. This does not seem to have been observed in other Cryptomonadinae and the flagellate under consideration would be an excellent object for experimental study of the co-ordination of cell-organs during division. Only the variation as observed in the living cell is described, as no stained material was studied. Chromatophore and contractile vacuole situated in the anterior end of the cell seem to divide simultaneously (Fig. 4*a*). The pyrenoid exhibits a varied behaviour; thus in Fig. 4*a* it has not divided, although in Fig. 5*a*, where chromatophore and vacuole are as yet undivided, division of the pyrenoid has occurred. In Fig. 5*b* all three organs have not yet divided, while the trichocysts—and with them the furrow—have completed division. An earlier stage of separation of the trichocysts is seen in Fig. 5*a*.

As regards the eyespot, two distinct pigment granules can be seen in cells where the chromatophore is in an advanced stage of division (Fig. 4a), but also in those in which the former shows no change at all. On the other hand, there is an undoubted correlation between the division of the pyrenoid and the stigma, since in most cases the latter consists of two parts, or actually of two separate entities when the pyrenoid is dividing (Figs. 5a, 6c, d). Exceptions are shown in Fig. 4a with two and Fig. 6e with one granule respectively, although in the latter a split in the pigment granule might possibly be overlooked owing to its position. In dividing pyrenoids (Figs. 6a-e) two starch envelopes are visible; these are found with rare exceptions, such as Figs. 5a and 5b. The starch is sometimes found in course of solution prior to cell division, but this seems to be unusual. In young daughter-cells the pyrenoid sometimes possesses only a single starch envelope (Fig. 5c, on the right), although formation of the second seems to follow very quickly, as such stages are infrequent. The relative position of the two individuals in Fig. 5c is noteworthy, since one must have turned through 180° after division; this suggests a considerable amount of amoeboid movement during the palmelloid stage.

The above facts and figures are given in some detail, as little information on these matters is to be found in the literature. Since Pascher's monograph in 1913 there has been no comprehensive treatment of the Cryptomonadinae and the number of original papers is very small. Division has been described by Belar (1916), Reichardt (1927), and Dangeard (1910), but our knowledge is still very incomplete. No figures of division in blue-green Cryptomonads have been published.

In 1885 Hansgirg described a genus *Chroomonas* with the species *C. Nordstedtii* of which a detailed Latin diagnosis is given without drawings. This species has since been reported from many localities, but the only illustration is that of Pascher (1913, p. 104), which is rather diagrammatic and does not show certain structures referred to by Hansgirg. Hansgirg's diagnosis agrees in all essential respects with the Windermere form, although he does not definitely mention the presence of a stigma. The passage in his diagnosis reads: '*Chroomonas* . . . corpusculis chromatinicis pluribus, exiguis, praedita.' As it is improbable that these words refer to a chromatin structure, it is suggested that Hansgirg is referring to the small pigment granules in the centre of the cell which were interpreted above as the stigma. It is open to discussion whether these striking granules can really be homologized with a stigma, which is usually located near the anterior end and in the superficial layer of the cytoplasm. The relation to the chromatophore which obtains for certain classes does not hold for all (e.g. Eugleninae), and this may also be so



FIGS. 6a-e. *Chroomonas Nordstedtii* Hansg. Division stages of pyrenoid; close correlation of stigma and pyrenoid; s, starch.

in Cryptomonads. Fritsch (1935) refers to *Nephroselmis* as the only member of the Cryptophyceae which possesses an eyespot, but there are two other statements in the literature as to the occurrence of eyespots in Cryptomonads. Geitler (1922) described *Cryptomonas coerulea* with one almost central stigma near the pyrenoid, and Wislouch (1924) states of *Cryptomonas stigmatica*: '... in parte centrali media stigmatem uno, pallido lutea, reniformis,' referred to as 'orange' in the Polish text. Carter (1938) refers to the same species as having an inconspicuous eyespot.

Both statements agree with the present observations, and together they afford evidence of the occurrence of a stigma in the true Cryptomonads. The presence of a stigma already established by Hansgirg in *Chroomonas* should be added to the diagnosis given by Pascher.

In 1894 Davis published an account of *Cryptoglena americana* which, as will be shown, is identical with *Chroomonas Nordstedtii*. Oltmanns (1904) recognized that this organism was really a Cryptomonad to which he gave the name *Cyanomonas americana*, although he added no new observations. The figures he gives, which are said to be reproduced from Davis's paper and are repeated in the second edition of 1922, appear to have been fundamentally altered in the process of redrawing. The light and dark shadings have been reversed so that the discoid bodies drawn light on a dark background by Davis are now dark on a light background; this gives the impression of several discoid chromatophores of various sizes. In addition the bacteria drawn by Davis in Fig. 2, to show the edge of the palmella stage, have been reproduced as minute dots. Oltmanns' version of the figures was copied into other well-known books and accepted as representing the real structure of the flagellate. The number and shape of the chromatophores, as well as the pigment granules in the centre of the cell, were given by Davis and later by Pascher as the chief points of distinction from other blue-green flagellates, but Davis's figures do not justify this claim.

Assuming that they are the correct representation of what he observed, a comparison of Davis's figures with the present observations lead one to conclude that he misinterpreted his own figures on Plate XI. Fifty-five motile and non-motile cells are drawn, all in about the same state. They correspond to the cells shown in my Fig. 1, with the chromatophore hardly visible, except for the dark background it provides for the closely packed globular bodies of starch. The edge of the chromatophore is actually visible at a few points in Davis's Fig. 1. In 41 of the 55 cells shown he figures a single central pigment granule, while in 4 cells there are 2 and in 1 cell 3. According to the interpretation given above, these supernumerary granules are the result of early division of the eyespot, although the cell with three granules is puzzling and is perhaps an abnormal case of successive divisions. In several cells a large light body at the posterior end corresponds in position and size to the pyrenoid, which is, however, not mentioned by Davis. He seems to interpret this structure as the nucleus, which is only visible after special treatment. Various tests, which later work has shown to be unreliable,

induced Davis to conclude that there were 6 to 10 'disc-shaped' chromatophores. He used 'dilute potassic hydrate solution' after treatment with mercuric chloride in absolute alcohol, and found that the bodies in question were readily destroyed, which was taken as proof that they were chromatophores. In his Fig. 4 cells treated with absolute alcohol show up to ten bodies of quite irregular shape which are again interpreted as chromatophores. Davis's remarks on page 97—'For this reason it seems as though these bodies are true chromatophores, although the blue-green tint is not always confined to them. Sometimes the blue-green color seems to fill almost the entire cell, only the end which bears the cilia being hyaline'—make it quite clear that he was under a misapprehension in interpreting his drawings.

No contractile vacuole is mentioned or figured, but it may be assumed that it was overlooked in view of the date of publication and the physiological state of the cells.

Another discrepancy between my observations and those of other authors is the presence of trichocysts (Figs. 3 and 5). The different figures given in this paper show that the trichocysts are not equally well visible in all stages and may readily have been overlooked if material in only one physiological state was examined. Pascher (1913, p. 104) figures *Chroomonas pulex* with some trichocysts along the furrow, so that their presence has already been established for the genus. The arrangement and number of the trichocysts in Geitler's *Cryptomonas coerulea*, referred to below, is identical with that in *Chroomonas Nordstedtii*. Carter (1938) refers to the difficulty of seeing the gullet and granulation in the new species *Chroomonas vectensis*, which is another blue-green Cryptomonad.

The foregoing discussion shows that the form described and figured by Davis must be included in *Chroomonas Nordstedtii*, with *Cyanomonas americana* as a synonym. A note in the 'Süsswasserflora' seems to indicate that Pascher has observed blue-green Cryptomonads with several chromatophores; when such forms are fully described and figured they will have to receive a new name.

It is difficult to assess the position or validity of the forms described by Dangeard (1890, 1910), as very incomplete drawings accompany them and the descriptions are insufficient to indicate what was really seen. The measurements given, however, make it unlikely that any of Dangeard's species could be identical with the form under discussion.

The blue-green *Cryptomonas coerulea* described by Geitler (1922) is, however, strikingly similar to *Chroomonas Nordstedtii*. The only differences are the presence of a gullet in Geitler's form, although this is not convincingly figured, and the equal length of the flagella. The shape and structure of the cell, the presence and position of the stigma, as well as the dimensions are, however, so similar in the two species that the possibility of their identity cannot be excluded. The somewhat similar habitat in the littoral region of lakes lends further support to this possibility. Geitler does not draw attention to this similarity, although he records *Chroomonas Nordstedtii* in the same

habitat. Future work, especially on the significance of the gullet, will have to show what the relation between these forms is.

The dimensions given by the various authors are as follows: Hansgirg:  $9-12\ \mu \times 6-8\ \mu$ ; Davis: motile cells  $8-10\ \mu \times 5-6\ \mu$ , non-motile cells  $7-9\ \mu \times 6-7\ \mu$ ; Rosenberg:  $7-9.8\ \mu \times 4.2-7\ \mu$ ; Geitler for *Cryptomonas coerulea*  $8-10\ \mu \times 6-7\ \mu$ .

*Chroomonas Nordstedtii* is the only species of the genus recorded for Great Britain and has been found only once in Harborne, Worcestershire (Grove, 1920).

The two other species, *C. pulex* Pascher and *C. caudata* Geitler (1924), are sufficiently different not to be confused with the form described here.

The foregoing matter shows clearly that a revision and monograph of the Cryptomonads is urgently needed to clear up the many mistakes and misinterpretations which have been carried through the literature far too long.

#### SUMMARY

The Cryptomonad *Chroomonas Nordstedtii* is described and figured, and various additions to Hansgirg's original diagnosis are made. Considerable evidence is produced for the view that Davis's *Cryptoglana americana* should be considered as identical with *Chroomonas Nordstedtii*; his figures, which differ very much from his description, support this view. Attention is drawn to the close resemblance between *C. Nordstedtii* and *Cryptomonas coerulea* Geitler. It seems very doubtful whether there was any justification for the establishment of the genus Cyanomonas by Oltmanns, and it is suggested that this should be cancelled since there is no species described or figured which corresponds to the diagnosis.

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# Physiological Studies in Plant Nutrition

## XI. The Effect on Growth of Rubidium with low Potassium Supply, and Modification of this Effect by other Nutrients

### PART II. THE EFFECT ON DRY-WEIGHT DISTRIBUTION, NET ASSIMILATION RATE, TILLERING, FERTILITY, ETC.

BY

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With eight Figures in the Text

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## INTRODUCTION

THE first part of this paper (Richards, 1941a) was concerned with the description and elucidation of the effects on dry-weight increase found in an experiment in which barley was grown under twenty nutritional treatments. The data dealing with the partitioning of dry weight will now be reviewed, together with such variables as net assimilation rate, tiller production, water content, and fertility, and the relationships between them. Attention will be directed mainly to effects of rubidium addition.

The plants were grown in the presence and absence of rubidium at two potassium levels, both relatively low, and at two phosphorus levels, one low and one very high. The eight possible combinations of these three factors were also combined factorially with two diverse types of nutrient solution, one containing nitrate and phosphate in the form of calcium salts and the other as ammonium salts, so that in the former calcium was present in excess and in the latter was at a low level though sufficiently abundant to maintain

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normal growth at high potassium and phosphorus levels. A basal solution of intermediate composition was also used with the four potassium and rubidium combinations at the high phosphorus level. For details of the nutritional scheme, experimental technique, dates of sampling, &c., reference must be made to the earlier paper (Richards, 1941a), but it may be convenient to reproduce here a table of the nomenclature scheme for the twenty treatments (Table I).

TABLE I  
*Nomenclature*

|                              | High ammonium   |                  | High calcium    |                  | Intermediate ammonium and calcium |
|------------------------------|-----------------|------------------|-----------------|------------------|-----------------------------------|
|                              | Low phosphorus. | High phosphorus. | Low phosphorus. | High phosphorus. | High phosphorus.                  |
| Low potassium                | M : O           | M : P            | C : O           | C : P            | X : P                             |
| High potassium               | M : K           | M : PK           | C : K           | C : PK           | X : PK                            |
| Low potassium<br>+ rubidium  | M : R           | M : PR           | C : R           | C : PR           | X : PR                            |
| High potassium<br>+ rubidium | M : KR          | M : PKR          | C : KR          | C : PKR          | X : PKR                           |

#### EXPERIMENTAL RESULTS

##### *Distribution of dry matter among plant parts*

(a) *Series C.* The most important effects on weight distribution of the eight treatments in series C at the time of sample 3 are presented in highly condensed diagrammatic form in Fig. 1. Since the main effects and interactions at the time of sample 2 are closely similar to those at sample 3 they are not presented; at sample 1 the percentage of dead tops is very low everywhere, but again the percentage roots are distributed similarly to those in sample 3.

The diagram itself needs some explanation. It combines the method of representation using triangular coordinates with that of the factorial diagrams used elsewhere in this paper. The three variables root weight, living-tops weight, and dead-tops weight are expressed as percentages of total dry weight, and the eight treatment points are inserted in a triangular diagram in the usual manner. The points are interconnected by a network of lines, one type of line for each nutritional factor involved; by bisection of treatment lines and diagonals of quadrilaterals the various treatment effects and interactions are deduced. This is accomplished just as in the diagram illustrating effects on a single variate in a factorial experiment (Richards, 1941, 1941a), the only difference being that the positions of the eight experimental points are now determined directly by the observational data and thus cannot be rearranged or grouped systematically in any direction. The diagram therefore loses much in orderly arrangement, and, though basically similar to the simpler diagram, requires closer scrutiny in its interpretation. This disadvantage is offset by the much greater amount of information contributed by the same



amount of movement necessary is twice the length representing the magnitude of the interactions in the other two variates. This condition is nearly fulfilled by the  $P \times R$  interaction in Fig. 1, *vide* the mid-points of the potassium (white) constructional lines.

It is simpler of course to bisect the diagonals of the figure and represent the interaction relative to all three variates by a single vector passing through the general mean. In the present instance we obtain the vector  $pr$ , which is seen to be nearly parallel to the percentage roots base. The lengths of three projections of such a vector may be directly read off the three coordinate scales, thus giving the magnitudes of the three interactions represented by the vector. The direction indicated by the vector may also be interpreted relative to all three sides of the triangle. Thus, moving along the  $pr$  vector in the direction of the arrow-head we recede from the sides representing respectively the bases of percentage roots and percentage living tops and approach the percentage dead-tops base; the  $P \times R$  interactions in percentage roots and living tops are therefore positive, while that in percentage dead tops is negative, the summation of all three necessarily being zero. The three magnitudes thus obtainable from any one vector may be compared with those shown at the sides of the diagram, which represent the lengths required for the effects under consideration to be significant either at the 5 per cent. or 1 per cent. level.

The vector representing the  $P \times K \times R$  interactions ( $pkr$ ) and all main effects of the three nutritional factors may be obtained geometrically exactly as in the simpler factorial diagrams, and are interpreted in the same manner as those representing the first-order effects. In Fig. 1 the second-order interactions have been reduced to a single vector passing through the general mean, and all main effects are represented by two points; thus the point  $-p$  is the mean of the four treatments at the low phosphorus level, and  $+p$  is the mean of the corresponding four at the high level. The two points therefore mark the ends of a vector again passing through the general mean. Finally, each of the twelve constructional lines connecting the eight experimental points is itself a vector, which may be interpreted relative to all three sides of the triangle.

The estimates of error given in Fig. 1 appear to be reliable. In spite of the fact that the percentages of dead tops are very low in some treatments the observed variances here are no lower than where the proportion of dead matter is higher. Of the seven treatment degrees of freedom relative to percentage roots, one only is significant, that representing the phosphorus effect, whereas of the seven relative to living tops five attain significance, four of them exceeding their 1 per cent. level; in percentage dead tops all effects with the exception of the  $P \times K$  interaction are highly significant, exceeding their 1 per cent. level. The paucity of significant effects in percentage roots is not due to larger errors, since the errors in percentage living tops are quite as great; and indeed the diagram shows immediately that, with the exception of the vector representing the phosphorus effect, all the others, representing both main and interaction effects, run nearly parallel to the

percentage roots base; hence effects on percentage roots are generally small compared with those on living and dead tops.

The main phosphorus vector itself runs approximately at right angles to the roots base, indicating nearly equal increases in percentage living and dead tops accompanying a relative decrease in roots with increased phosphorus level, i.e. the *average* effect of phosphorus is almost entirely confined to the root : top ratio, scarcely affecting senescence of the aerial parts. This statement needs amplifying since there are large and highly significant interaction effects of phosphorus with rubidium in both the aerial fractions. Thus, increasing the phosphorus level in the absence of rubidium (black lines connecting treatment C : O with C : P, and treatment C : K with C : PK) does not change the percentage living tops, the loss in relative root weight reappearing as an equal gain in relative weight of dead tops; but increasing the phosphorus level in the presence of rubidium (lines connecting C : R with C : PR, and C : KR with C : PKR) leaves the percentage dead tops almost unchanged, and the reduced relative root weight is balanced by a relative gain in living tops. Increase in phosphorus level then always reduces considerably the percentage roots; in the absence of rubidium, phosphorus adversely affects survival of the tops, but with rubidium present it has no such effect. The  $P \times K$  interaction is negligible in all plant fractions, and the second-order effect ( $P \times K \times R$ ) is quite insignificant and negligible in percentage living tops and roots; although highly significant in percentage dead tops, the effect is not large (2.3 per cent.) and need not be discussed.

The main effects of potassium and rubidium are similar, survival of the tops being enhanced by either element. The effect is particularly large with rubidium, and indeed this is one of the outstanding results of application of that element. As was mentioned in the preceding paragraph, the rubidium effect depends largely on phosphorus level, being much greater at the high level than at the low. Rubidium and potassium also interact considerably and highly significantly, as might be expected, in both fractions of the tops. At the higher potassium level the beneficial effect of rubidium is reduced, while in the presence of rubidium potassium has no further appreciable effect. Rubidium moreover decreases the *absolute* amount of dead tops, while potassium invariably increases it, on account of the greatly increased growth induced. During the period covered by sampling, therefore, relative to potassium the effect of rubidium on the production of new leaf material is smaller, but its effect on leaf survival is larger.

(b) *Series M.* It was stated earlier that at sample 2 in series C the main effects and interactions are very similar to those described above for sample 3. The percentage dead tops are at this time considerably lower than at sample 3, the deficit being balanced mainly by higher percentage roots. At sample 1 percentage roots are still higher, i.e. the root : top ratio falls continuously from sample 1 to 3. A similar fall in the ratio value is characteristic also of series M, although as usual treatments M : O and M : P are aberrant, maintaining the high values of sample 1 throughout the sampling period. This is probably

accounted for largely by the retardation in development resulting from these treatments; by the final harvest the root : top ratios have fallen to low values, though they are still considerably higher than the final values reached in other treatments. Treatments M : O and M : P have likewise high percentage dead tops (nearly 30 per cent.), and differ from other treatments again in that these values are high even at sample 1.

As regards the percentage values of the three plant fractions under consideration these two treatments (M : O and M : P) are very much more variable than the others, hence estimates and tests of significance of the statistical effects within series M are of doubtful value. The effects are in any event very largely determined by the two most variable treatments, which in a triangular diagram are much farther removed from the other six treatment points than are C : O and C : P in Fig. 1. Five of these other points are disposed almost exactly as in series C, the remaining one, M : PKR, being situated farther from the percentage roots base in the direction of treatments KR and R.

There is, however, clear evidence to show that the effects are in general similar to those within series C. The largest effects are those of potassium and rubidium, and of their mutual interaction, in the variates percentage living and dead tops, and these effects must be accounted real. All six are numerically greater than their counterparts in series C and are always of the same sign. The phosphorus effect on percentage roots is smaller than that in series C, and in view of the variability cannot be adjudged significant in this series without supporting evidence; but the effect is otherwise similar to that in series C and is presumably real. The only other effect in series M large enough to be unquestionably real is the  $K \times R$  interaction on roots; the vector representing this interaction in a triangular diagram is considerably larger than the corresponding vector in Fig. 1 and is rather more steeply inclined to the percentage roots base. The interaction is otherwise similar in both series.

(c) *Series X.* Finally, in series X, as regards position within the triangular diagram three of the four treatments agree closely with the corresponding treatments of both series M and C, the fourth (X : PKR) being intermediate in position between the positions of the similar treatments in series M and C.

#### *Net assimilation rate*

The calculated net assimilation rates for the two inter-sampling periods are shown in Table II; since obviously there are large interaction effects and there is no independent estimate of error, the main results will be only briefly indicated.

Effects of phosphorus are usually small though its addition to treatment C : O leads to a reduction from the earliest sample, with the result that growth rate in C : P is lower than in C : O, and moreover eventually lower than in any other treatment from the series except C : R. Between samples 1 and 2

assimilation rate is as high in treatment PK of both series M and C as in treatment K, but after sample 2 in both series treatment PK declines, the fall in M : PK being very rapid. This change is accompanied by death of many

TABLE II  
*Net Assimilation Rate (gm. per sq. dm. per week)*

| Treatment. | First period. |           |           | Second period. |           |           |
|------------|---------------|-----------|-----------|----------------|-----------|-----------|
|            | Series M.     | Series C. | Series X. | Series M.      | Series C. | Series X. |
| O          | 0·14          | 0·65      | —         | 0·09           | 0·38      | —         |
| P          | 0·08          | 0·30      | 0·31      | 0·16           | 0·29      | 0·24      |
| K          | 0·53          | 0·45      | —         | 0·36           | 0·50      | —         |
| R          | 0·42          | 0·43      | —         | 0·38           | 0·25      | —         |
| PK         | 0·50          | 0·57      | 0·51      | 0·19           | 0·39      | 0·23      |
| PR         | 0·35          | 0·50      | 0·41      | 0·44           | 0·25      | 0·41      |
| KR         | 0·47          | 0·24      | —         | 0·44           | 0·48      | —         |
| PKR        | 0·48          | 0·49      | 0·60      | 0·54           | 0·53      | 0·41      |

of the leaves, the resultant effect on growth being disastrous. There appears to be a small but fairly consistent rise in assimilation rate from treatment KR to PKR.

Effects of potassium are always positive in the M series, those in the absence of rubidium being usually large. In C series at the lower phosphorus level potassium has little effect when averaged over the whole four weeks, but at the higher level (C : P to C : PK and C : PR to C : PKR) it appears to be definitely beneficial.

Rubidium leads to a large increase in assimilation rate at the low potassium level in M series (M : O to M : R and M : P to M : PR); this increase is not so great as the corresponding one due to potassium (M : O to M : K and M : P to M : PK) in the first period, but is greater in the second, since with potassium the rate falls rapidly with time while with rubidium it is maintained much later. Addition of rubidium to treatment M : K leads to no appreciable change in the rate, nor does its addition to M : PK in the first period; but again M : PKR maintains its rate through the second period whereas M : PK declines greatly. In C series the addition of rubidium at the lower phosphorus level is always detrimental, but on the average at the higher level it appears to be slightly beneficial.

Just as with dry-weight increase, net assimilation rates in the four treatments with both calcium and ammonium (X series) at the high phosphorus level are generally intermediate between those of corresponding treatments from the M and C series, and always so if averaged over the two fortnightly periods.

#### *Water content*

The results of an analysis of the water content data are presented in Tables III and IV, where the calculated statistical effects and their estimated

TABLE III

*Statistical Effects derived from Water Content Data of green Leaves*  
(% dry weight)

| Effect         | Series M. |           |           | Series C. |           |           |
|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                | Sample 1. | Sample 2. | Sample 3. | Sample 1. | Sample 2. | Sample 3. |
| General mean   | 519       | 581       | 501       | 472       | 511       | 441       |
| P              | +60.7     | +106.1    | +108.4    | -11.6     | +16.8     | +39.4     |
| K              | -51.2     | -76.3     | -8.9      | -0.9      | +19.1     | -54.5     |
| R              | -19.8     | -76.6     | -70.3     | +21.2     | -12.6     | -18.0     |
| PK             | -13.9     | -42.1     | +57.3     | -21.2     | -42.4     | -22.0     |
| PR             | +8.2      | +7.6      | +11.6     | -17.1     | -3.4      | -19.5     |
| KR             | +19.3     | +57.3     | +0.9      | -1.4      | +22.0     | -10.7     |
| PKR            | +13.3     | +11.1     | -30.6     | +5.3      | -5.3      | -0.9      |
| Standard error | ±11.47    | ±25.65    | ±15.41    | ±8.80     | ±12.90    | ±14.03    |
| 5%             | 24.4      | 54.4      | 32.7      | 18.7      | 27.3      | 29.7      |
| 1%             | 33.8      | 74.9      | 45.0      | 25.7      | 37.7      | 41.0      |

Effects in italics significant beyond the 5 per cent. level.

Effects in heavy type significant beyond the 1 per cent. level.

TABLE IV

*Statistical Effects derived from Water Content Data of 'Stems'*  
(% dry weight)

| Effect         | Series M. |           |           | Series C. |           |           |
|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                | Sample 1. | Sample 2. | Sample 3. | Sample 1. | Sample 2. | Sample 3. |
| General mean   | 807       | 791       | 660       | 641       | 709       | 535       |
| P              | +128.4    | +145.3    | +246.3    | +101.7    | +30.0     | +78.0     |
| K              | -39.1     | -13.4     | +10.2     | +66.5     | +58.3     | -73.8     |
| R              | -152.3    | -141.7    | -197.5    | -81.9     | -174.9    | -170.0    |
| PK             | +5.9      | -18.3     | +75.8     | -25.4     | -68.5     | -48.4     |
| PR             | +54.8     | +43.7     | -21.8     | +3.0      | +34.3     | -50.2     |
| KR             | +52.0     | +31.7     | +36.6     | +19.6     | +120.0    | +97.4     |
| PKR            | -64.8     | -39.0     | -30.4     | +3.0      | -13.2     | +10.5     |
| Standard error | ±28.43    | ±33.86    | ±31.04    | ±10.35    | ±25.01    | ±25.10    |
| 5%             | 60.3      | 71.8      | 65.8      | 21.9      | 53.0      | 53.2      |
| 1%             | 83.0      | 98.9      | 90.7      | 30.2      | 73.1      | 73.3      |

Effects in italics significant beyond the 5 per cent. level.

Effects in heavy type significant beyond the 1 per cent. level.

errors are given. In addition the mean water contents of the 'stems' derived from all three samples are presented as factorial diagrams in Figs. 2 and 3. For simplicity in the statistical analysis the two main series are dealt with separately, at each sampling time. A more comprehensive analysis reveals a highly significant complex interaction between all four nutritional factors, and several significant second-order effects involving sampling occasions and the comparison between the M and C types of nutrient. These, however,

will be referred to only in a general manner. The green leaf and 'stem' data are presented separately. Water contents within the X series are again intermediate between those of the C and M series and are not presented.

It may be seen immediately that variation in water content due to treatment is generally greater in 'stems' than in leaves; this is particularly evident in series C, in which the leaves are highly resistant to such changes. Correlated

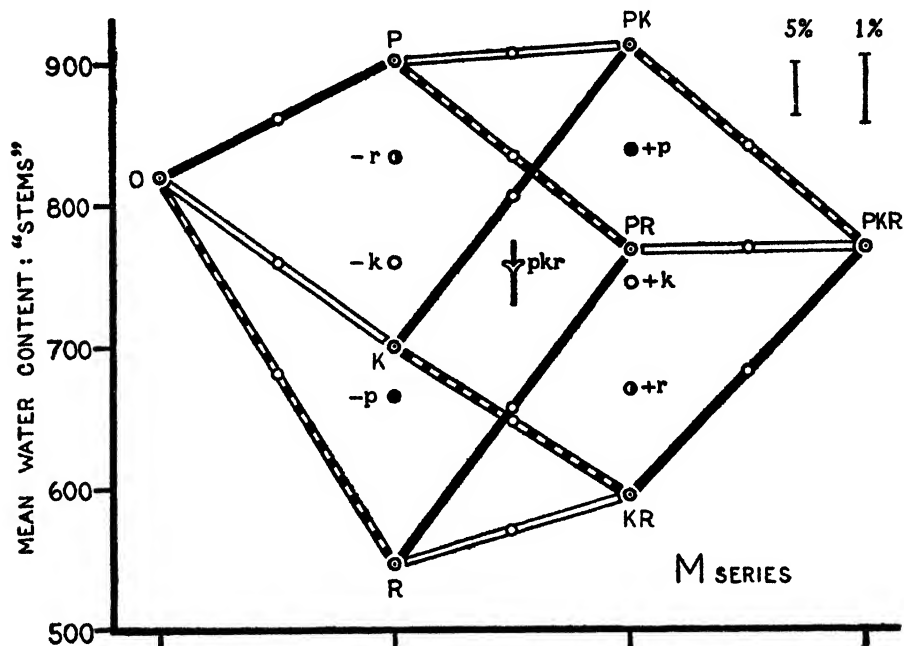


FIG. 2. Interaction diagram for mean water content over all sampling occasions of 'stems' of series M. Black lines represent a difference of phosphorus level, white lines one of potassium level, and pied lines one of rubidium application. The main effect of each of the three nutrient variables is represented by two points giving the mean values at the high and low levels of the element.

with this is the fact that the general mean water content is lowest in the leaves of C, higher in those of M, and highest in 'stems' of M.

With the exception of an insignificant negative value for the leaves of series C at sample 1, the phosphorus main effect is always positive and in nine out of the twelve analyses is highly significant. The main effect of rubidium is as consistently negative, eight of the analyses showing very high significance; the single exception is provided again by the leaves of series C at sample 1, where a moderate positive value, significant at the 5 per cent. level, is found. Interaction between phosphorus and rubidium results in both positive and negative effects which are never sufficiently large to be demonstrably real. Again, the second-order interaction involving potassium as well as phosphorus and rubidium exceeds its 5 per cent. point once only in twelve determinations, so that of the total twenty-four examples of inter-



action involving the two elements under consideration, one only is found slightly greater in magnitude than its 5 per cent. value, a result which on this score alone might well be attributed to random causes. It will be referred to more fully later. In the experiment, therefore, the total effects of phosphorus and rubidium supply are clear cut and mainly simple; they are always opposed and largely independent.

Effects of potassium are not so simple. Three highly significant main

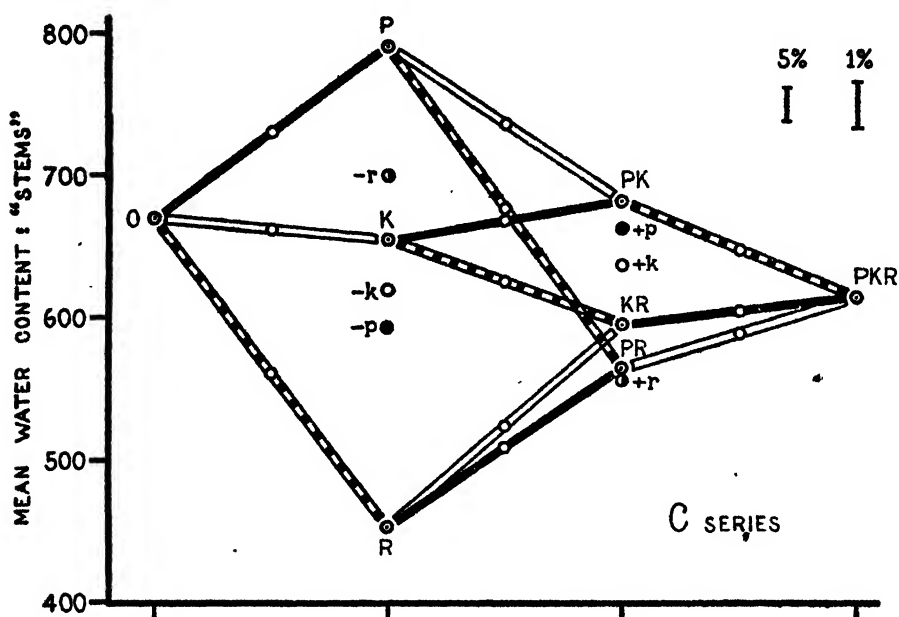


FIG. 3. Interaction diagram for mean water content over all sampling occasions of 'stems' of series C. Black lines represent a difference of phosphorus level, white lines one of potassium level, and dashed lines one of rubidium application. The main effect of each of the three nutrient variables is represented by two points giving the mean values at the high and low levels of the element.

effects are found in the leaf data, all of them negative. In the 'stem' data of series M the main effects are always slight, while in those of series C the three effects are all highly significant, being positive at samples 1 and 2 and negative at sample 3, so that in the data averaged over all samples the effects cancel out. In leaves and 'stems' of series M the  $P \times K$  interaction appears usually negative in the first two samples, though insignificantly so, while in the last it is large, highly significant, and positive. The change is due primarily to abnormal behaviour at this stage of plants from treatment M: PK; as has been mentioned on other occasions, rapid deterioration set in here after sample 2, and accompanying this the water content remained high, or even rose, instead of declining as in other treatments with the lengthening of the stems. In the C series general deterioration of plants from the corresponding treatment occurred later (although the actual percentage of com-

pletely dead matter was as high at sample 3 as in series M), and the accompanying relative rise in water content was comparatively small up to the time of sample 3. On the contrary, series C : P was deteriorating rapidly relative to the other treatments throughout the sampling period (cf. Part I), and this deterioration was again accompanied by a large relative rise in water content. Consequently the  $P \times K$  interaction in leaves and 'stems' of the C series remains negative over the whole sampling period, though it is significant in the first two samples only. In series M, treatment M : P likewise had a high water content from the beginning, accounting for the negative  $P \times K$  interaction in earlier stages. It may be mentioned that the effects ascribable to phosphorus, potassium, and their interaction, in series C, are closely similar to those found previously (Richards and Shih, 1940).

The  $K \times R$  interaction, when of any magnitude meriting attention, is invariably positive. In series M significance at the 5 per cent. level is attained once only, in the leaf data of sample 2, but much larger and highly significant results appear in the 'stem' data of series C at samples 2 and 3. Where significant, this positive interaction is always associated with negative rubidium effects, so that the effect of rubidium is smaller at the high potassium level than at the low, just as in the C series the positive effect of phosphorus is reduced at the high potassium level. In these instances too, potassium in the absence of rubidium has a negative effect, but in its presence the potassium effect is reduced to negligible dimensions, or may even assume a large magnitude in a positive direction ('stems' of C, sample 2). A fairly general effect of potassium in reducing the effects of other mineral elements on water content, similar to that just described, was discussed by Richards and Shih (1940).

The second-order interaction, referred to above, while significant only in the 'stem' fraction of series M at sample 1, is presumably real here over the whole sampling period. At both later samples the effect is rather large and its components are completely consistent at all sampling times, comprising a large positive  $K \times R$  interaction at the low phosphorus level together with no evidence of interaction at the high phosphorus level. The mean effect over all three samples is highly significant. In the 'stems' of series M therefore the  $K \times R$  interaction is of the same kind and similar in magnitude to the highly significant interaction found in 'stems' of series C, but is confined to treatments at the lower phosphorus level. The  $P \times K \times R$  interaction in the leaves of series M at sample 3 almost attains its 5 per cent. level, and is again negative.

#### *Tiller number*

Tillering curves for all twenty treatments are presented in Figs. 4-6. Except for the data on the three sampling occasions, tiller counts are based on all available pots, excluding only those with no surviving plants. The usual effects of potassium and phosphorus are found, hence reference will be made almost exclusively to effects ascribable to rubidium. Of the ten treatments receiving rubidium, one only (M : KR) has fewer shoots at harvest than its

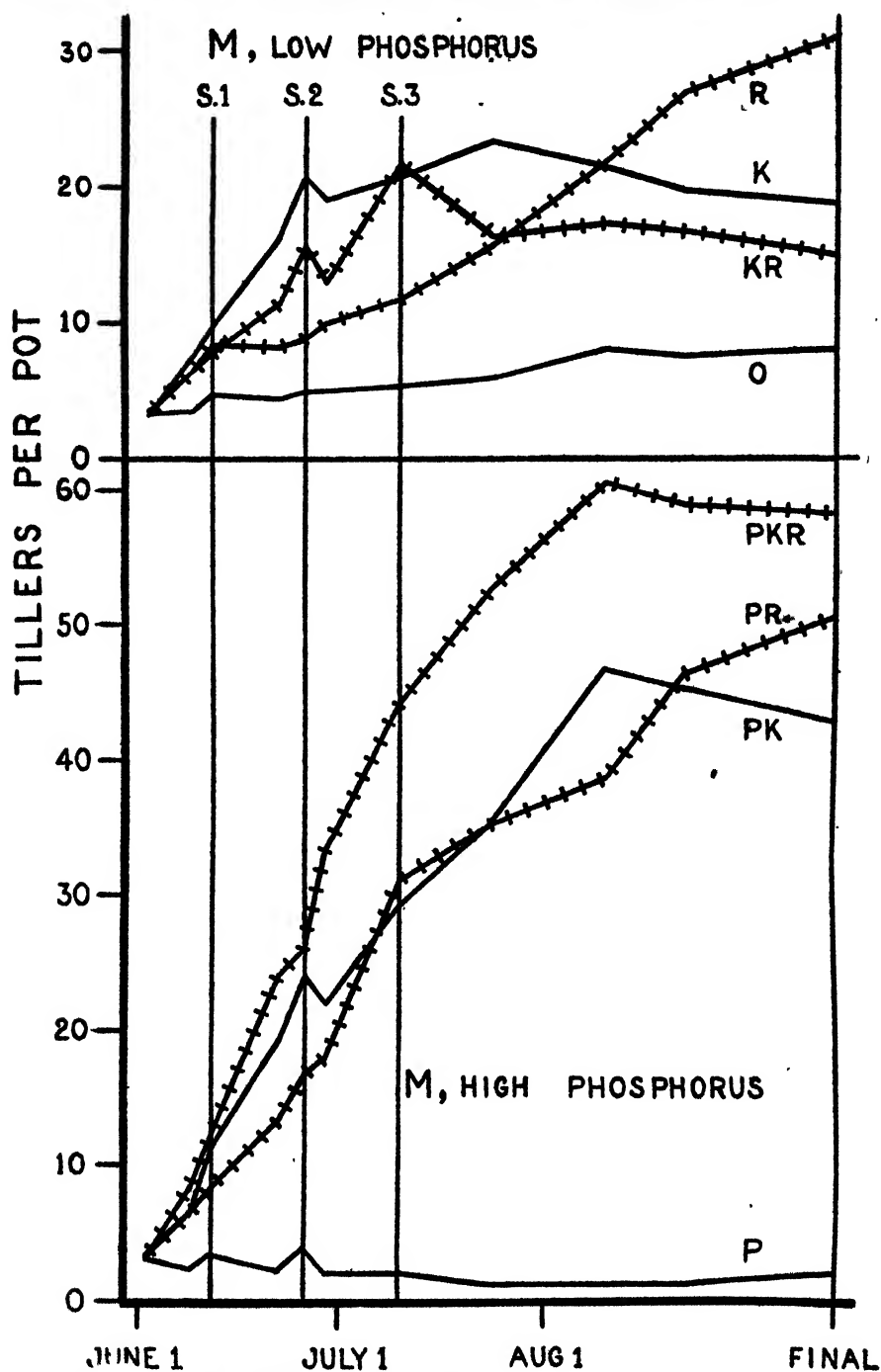


FIG. 4. Tillering curves for series M. The positions in time of the three sampling occasions are shown, but the position given to the final harvest count is arbitrary.

control without the element; in all other instances rubidium increases the final number of shoots, usually very considerably. In series C the rubidium effect found at harvest is always reversed in early stages; in the two comparisons with high phosphorus, plants with rubidium overtake their controls without it about the time of sample 2, while those from the corresponding two treatments receiving rubidium at the low phosphorus level do not overtake their controls until late in August. Of the intermediate series, a crossover between X : PKR and X : PK occurs rather earlier than in C series, while at the low potassium level X : PR has more tillers than X : P from the beginning. Of the two high phosphorus comparisons in the M series, plants from both treatments with rubidium are also in advance of their controls from the inception of tillering, the difference at the low potassium level being very great. The same is true also for the rubidium relationship at the low potassium and phosphorus levels of series M; but at the high potassium and low phosphorus levels (M : KR and M : K) rubidium treatment results in fewer shoots, even to harvest.

At the high phosphorus level therefore a progressive change in the rubidium effect is found from the C series, through the X to the M. The rubidium effect is always positive finally, but in early stages is positive in M, negative in C, and either positive or negative in X. At the high phosphorus and potassium levels, inception of a detrimental effect of rubidium on early tillering occurs in a basal treatment somewhere intermediate between M and X, while at the high phosphorus and low potassium levels it occurs towards the other end of the treatment range, between X and C.

The effect of reducing the phosphorus level is to reduce tiller numbers in treatments receiving rubidium to a considerably greater extent than in their controls without it. The very great difference between the curves for M : P and M : PR is much reduced at the low phosphorus level (M : O and M : R), and the similar effect at the high potassium level actually reduces M : KR to a level lower than that of M : K throughout life history. In the C series a precisely similar effect of lowering the phosphorus level is found, enhancing greatly the early reduction in tillering associated with rubidium treatment, and delaying the crossover until very late in life history.

A direct comparison of the relative effects of potassium and rubidium on tiller production may be made, since treatments R and K are presented with equivalent alkali metal. Potassium itself has a large positive effect in all five types of nutrient, the effect being in evidence from the inception of tillering. Rubidium effects have already been described; they are almost invariably positive finally, but in early stages may be positive or negative. The comparison (rubidium without potassium *v.* potassium without rubidium) also shows in general a greater final effect of rubidium than of potassium, though numerically the difference between the effects of the two elements is sometimes small; initially, however, potassium invariably produces greater tillering, and in C series this early superiority is considerable and persists until late in autumn.

It will be observed that in many instances the tillering curves, particularly for treatments receiving rubidium, do not as in more balanced solutions attain a maximum about the time of the later samples and thereafter decline somewhat, but take a rising course until late in the autumn. This course may

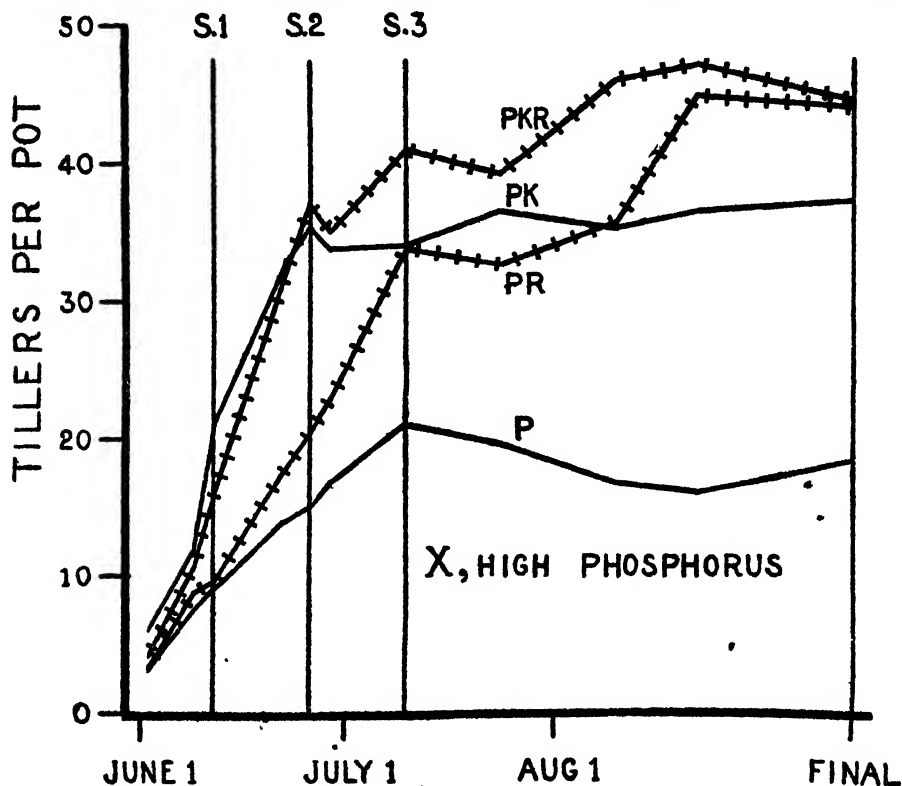


FIG. 5. Tillering curves for series X. The positions in time of the three sampling occasions are shown, but the position given to the final harvest count is arbitrary.

either be fairly regular, as in M : PKR, or give evidence of a double cycle of tiller production, as in C : PKR and C : PR.

#### *Ear and grain data*

The main final harvest data are presented in Table V. The whole experiment being conducted at potassium levels much below those required for maximal growth, ear production, and particularly grain production were in general poor. In many treatments, particularly those given rubidium at the high phosphorus level, tillering continued up to the time of harvesting. Here the earlier larger tillers usually produced ears and thereafter deteriorated rapidly, so that the ears and tillers bearing them did not ripen normally but rather died prematurely, often with the ears still completely enclosed within the leaf bases. Meanwhile fresh tillers were being rapidly produced; these

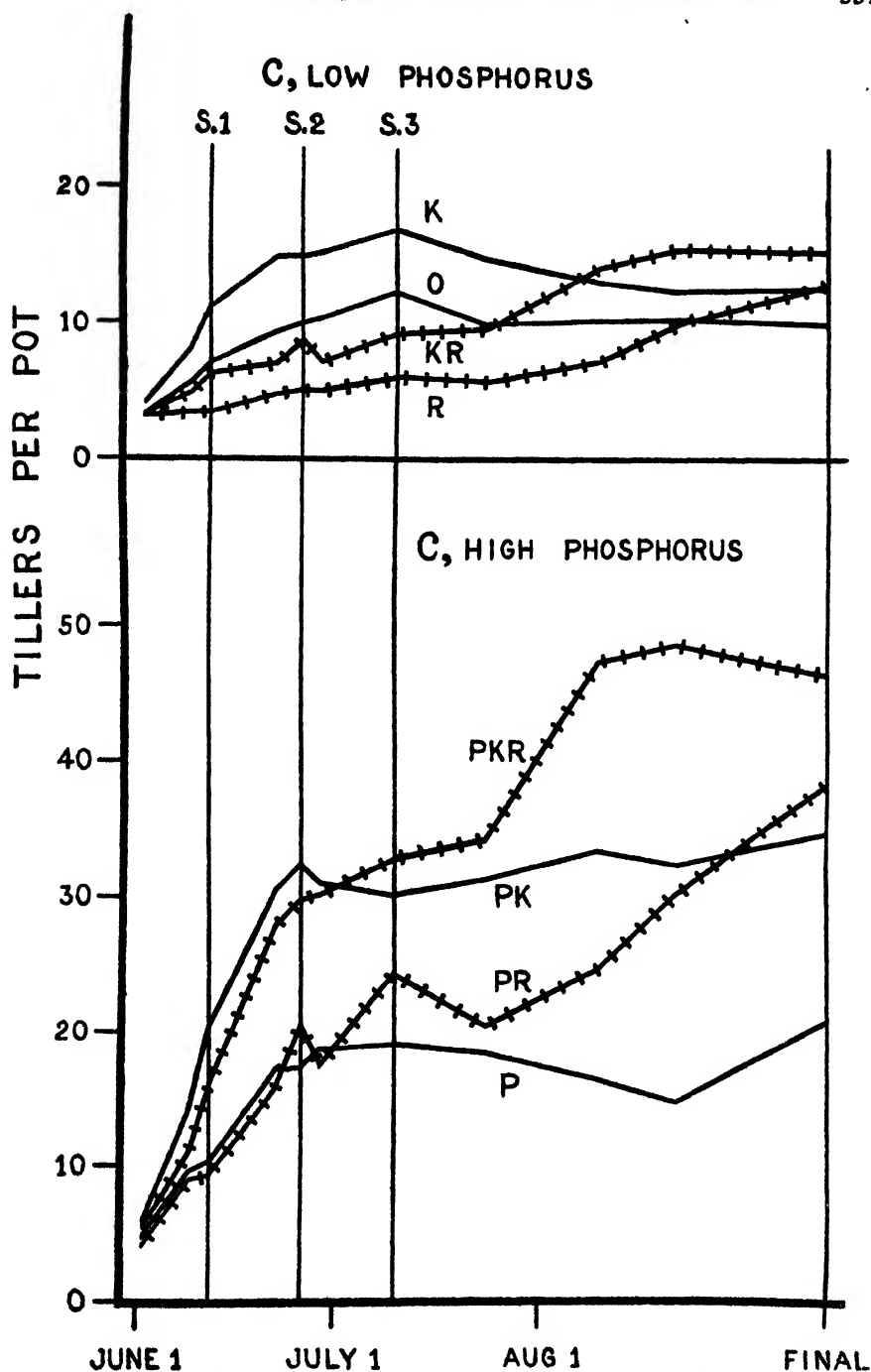


FIG. 6. Tillering curves for series C. The positions in time of the three sampling occasions are shown, but the position given to the final harvest count is arbitrary.

eventually produced a second crop of ears, again often with continued tillering at the base of the plants. At this stage the plants were harvested, since the season was too advanced to hope for any further ripening. Dates of harvesting the various treatments were given in tabular form in Part I, Table III.

TABLE V  
*Ear and Grain Data*

| Treatment. | Ratio<br>ear weight:<br>straw weight. | Per cent.<br>eared<br>shoots. | Weight<br>of ears<br>per pot. | Number<br>of ears<br>per pot. | Number<br>of fertile<br>grain<br>per ear. | Weight<br>of grain<br>per pot. | 1000-grain<br>weight. |
|------------|---------------------------------------|-------------------------------|-------------------------------|-------------------------------|---|--------------------------------|-----------------------|
| M: O       | 0.32                                  | 43.8                          | 0.87                          | 3.5                           | 3.1                                       | 0.38                           | 34.4                  |
| K          | 0.65                                  | 81.4                          | 6.55                          | 15.3                          | 8.9                                       | 4.18                           | 31.1                  |
| R          | 0.15                                  | 69.4                          | 1.03                          | 21.5                          | 0.0                                       | 0.02                           | 26.7                  |
| KR         | 0.73                                  | 88.0                          | 5.61                          | 13.2                          | 11.8                                      | 3.91                           | 25.6                  |
| M: P*      | 0.20                                  | 27.2                          | 0.60                          | 3.3                           | 2.2                                       | 0.11                           | 13.0                  |
| PK         | 0.29                                  | 66.7                          | 3.62                          | 28.7                          | 1.0                                       | 0.75                           | 29.2                  |
| PR         | 0.31                                  | 67.3                          | 3.25                          | 34.2                          | 0.1                                       | 0.06                           | 22.5                  |
| PKR        | 0.51                                  | 86.6                          | 9.96                          | 50.6                          | 4.0                                       | 3.58                           | 18.4                  |
| C: O       | 1.10                                  | 94.0                          | 4.99                          | 9.4                           | 13.2                                      | 3.37                           | 27.8                  |
| K          | 0.89                                  | 95.2                          | 7.80                          | 12.0                          | 14.4                                      | 5.74                           | 34.4                  |
| R          | 0.12                                  | 53.1                          | 0.30                          | 6.8                           | 0.0                                       | 0.00                           | —                     |
| KR         | 0.66                                  | 92.2                          | 4.59                          | 14.2                          | 7.3                                       | 2.64                           | 27.7                  |
| C: P       | 0.16                                  | 30.1                          | 0.75                          | 6.2                           | 1.4                                       | 0.32                           | 39.0                  |
| PK         | 0.49                                  | 72.8                          | 5.69                          | 25.2                          | 3.5                                       | 2.58                           | 28.3                  |
| PR         | 0.24                                  | 79.7                          | 2.84                          | 30.6                          | 0.0                                       | 0.07                           | 34.0                  |
| PKR        | 0.78                                  | 87.5                          | 15.48                         | 40.6                          | 12.0                                      | 9.89                           | 20.5                  |
| X: P       | 0.33                                  | 46.7                          | 1.84                          | 8.6                           | 2.1                                       | 0.69                           | 36.4                  |
| PK         | 0.30                                  | 70.1                          | 3.51                          | 26.2                          | 1.0                                       | 0.75                           | 29.8                  |
| PR         | 0.28                                  | 86.0                          | 3.93                          | 38.0                          | 0.1                                       | 0.08                           | 38.1                  |
| PKR        | 0.82                                  | 96.0                          | 16.08                         | 42.8                          | 11.1                                      | 10.59                          | 22.3                  |

\* Data derived from eight pots of an experiment in 1938.

At both phosphorus levels and the low potassium level, rubidium application results generally in greatly increased numbers of ears, the one exception being its addition to treatment C: O, which is followed by reduction in ear number. It usually leads also to reduction in ear size (individual ear weight, length of ear and awn) and deterioration in general appearance; sterility of the ears is nearly complete. At very low potassium levels then, treatment with rubidium always decreases the already low fertility of the ears to vanishing point, an effect not necessarily connected with the production of greatly increased numbers of ears. At the higher level of potassium and high levels of phosphorus, on the contrary, the presence of rubidium results not only in increased numbers of ears, but frequently also in larger, cleaner, and healthier ears and much greater fertility within the ear. Finally, at the higher level of potassium and the low level of phosphorus, rubidium has little effect in series M on ear number, weight, or fertility, though the last may be somewhat

increased; in series C, on the other hand, rubidium decreases considerably individual ear weight, fertility, and total grain weight.

The calculated mean statistical effects are presented in diagrammatic form in Fig. 7. Since no ears were produced by the single plant from treatment M : P surviving to the final harvest, figures have been provided from an experiment of the previous season. No fertile grain was produced in treatment C : R, hence no estimate of the 1,000-grain weight can be obtained; the statistical effects for this variate in series C have, however, been calculated on the assumption that the second-order interaction is zero, but the results are not presented. Large differences in variability are found among the various treatments, and standard errors may be quite misleading, hence none are presented; there is, however, no doubt about the reality of the larger effects.

Average effects of phosphorus are not generally very large, but are usually consistent between the M and C series. High phosphorus appears to reduce the percentage eared shoots (i.e. to encourage the production of late non-maturing tillers), the ear : straw ratio, and the number of fertile grains per ear. Although it may increase somewhat the weight of ears per pot, this is due entirely to the large increase in tillering and consequent large increase in numbers of ears, the average weight per ear being reduced. In series M, phosphorus reduces markedly the weight of the individual grains. These average effects of phosphorus are modified by large interaction effects, particularly with rubidium.

Main effects of potassium are almost invariably large, positive, and beneficial to fertility. The only exception to this generalization is found in the 1,000-grain weight, where there is little consistent effect.

Average effects of rubidium are not usually of a high order, though a large increase in the number of ears is found; there is also in the M series a considerable increase in the percentage of eared shoots. In general reduced grain size results from rubidium application.

Much the most important interactions studied are again those between phosphorus and rubidium in the C series. Effects here, except on grain size, are positive and very large, usually much larger than the main effects of either element. In the M series the corresponding interactions are much smaller, though again always positive. It is clear then, especially in the C series, that at reduced potassium levels high phosphorus supply is much more beneficial in the presence of rubidium than in its absence, or alternatively that rubidium is much more beneficial at high phosphorus levels than at low. The interactions of potassium supply with level of either of the other elements are of a lower order, though in the C series—again with the exception of grain size—they appear to be positive.

## DISCUSSION

*Net assimilation rate.* The observed differences in dry weight due to treatment, discussed in Part I, are mainly ascribable to differences in net assimilation rate, though the effect of variation in the proportion of total assimilate



converted into leaf material is sometimes important. Thus the rapid decline in dry weight of treatment C : P relative to C : O depends entirely on assimilation rate. The reduced growth rates of treatment C : R relative to C : O, and of C : KR relative to C : K, are also reflections of reduced assimilation rate. Similarly the assimilation rates of treatment C : PR relative to C : P, and of C : PKR relative to C : PK, and their changes with time, run closely parallel to the corresponding growth rate comparisons. Between samples 1 and 2 both assimilation and growth rates in treatment C : PR are considerably greater than in C : P, whereas between these treatments there is little difference in either process from sample 2 to 3; again, both rates are rather lower in C : PKR than in C : PK during the first interval, but during the second both are decisively higher in C : PKR. In relation to rubidium supply there is just as close a correspondence between estimated assimilation rate and response in growth throughout the M series.

Hence it is important that any hypothesis concerning the mechanism of the effects of rubidium on growth should be consistent with these effects originating primarily in antecedent effects on assimilation rate. It was suggested in Part I that within the experiment much of the effect of rubidium on growth is related to phosphorus nutrition, and may be largely accounted for in terms of the phosphorus contents of the plants; restriction of phosphorus uptake by rubidium leads to improvement where phosphorus is excessive, and to deterioration where phosphorus supply is low and already limiting growth. If this is largely a correct interpretation, effects of rubidium on growth may very well be secondary to assimilatory effects. For it is highly probable that assimilation rate rises with increasing phosphorus supply, other factors being constant, so long as the phosphorus can be efficiently metabolized, but when excess phosphorus accumulates it again declines; the critical external phosphorus level at which assimilation rate is maximal is not constant, but is determined by the levels of other factors. Evidence indicating such relationships has been obtained in work at this Institute.

The experiment of Shih (1936) supplies strong evidence of the deleterious effect on assimilation rate of excess phosphorus. He grew barley in twenty-two different nutrient solutions comprising three potassium levels (two identical with those of the present experiment and the third nine times the concentration of the higher) and two phosphorus levels, one being the same as the higher level of the present experiment and the second one-fifth of that level. At the high level of potassium there was no appreciable difference in net assimilation rate between the two phosphorus series, but at the two lower potassium levels, where internal accumulation of phosphorus occurred, every high phosphorus treatment had a considerably lower assimilation rate than its corresponding low phosphorus treatment. Up to the time of maximal leaf area the mean assimilation rate of eight treatments at low potassium and phosphorus levels, each measured in terms of the rate of a corresponding treatment at the high phosphorus level, was 1.23, while after the time of maximal leaf area the same comparison gave the high figure of 1.75, i.e.

increasing the phosphorus supply to levels above the optimum resulted in a mean decrease in net assimilation rate of 19 per cent. in early stages, and of 43 per cent. in later stages. It is interesting to note Shih's conclusion that excess phosphorus was responsible for much of the deterioration of his plants under potassium deficiency.

At the other extreme much reduced assimilation rates have been found in plants which respond to, and are deficient in, phosphorus. This effect has been observed on net assimilation rate in growth experiments, but has also been studied directly in the field, using gasometric methods.

*Tillering.* The interpretation of the observed effects of rubidium on tillering is not altogether clear, though most aspects appear to fit in with the following hypotheses: (1) there is a specific effect of rubidium tending to prolong and intensify tiller production, and (2) there are indirect effects consequent on retardation of phosphorus uptake. Phosphorus level is clearly one of the primary factors determining tillering rate in the experiment; hence if in general one effect of rubidium is to retard phosphorus uptake, a decreased rate of tillering may be expected, at least in earlier growth stages. The phosphorus analyses presented in Part I, together with the growth data, demonstrate a considerable reduction in phosphorus uptake due to rubidium throughout the sampling period in series C and X, but not in M; and tillering shows similarly a retardation in early stages in five out of the six available comparisons among the twelve treatments from the first two series, the exception being provided by the comparison between treatments X : P and X : PR. Moreover, if this is the correct interpretation of the reduced early tillering due to rubidium, the reduction should be greater at the low phosphorus level, where phosphorus is already limiting growth, than at the high level. Series C provides two comparisons of this kind, and in both it is obvious that the early reduction is much greater at the low phosphorus level than at the high and is continued to much later stages of life history.

The large early positive effect of rubidium found at the high phosphorus and low potassium levels in series M is much reduced in the X series and becomes negative in series C; at the high potassium level similar transitions are found from the M to the C series, but here the rubidium effect becomes negative at lower calcium, or higher ammonium, levels. At both potassium levels these gradations of the rubidium effect from the M series, through the X, to the C series, all at the high phosphorus level, appear to be further continued naturally to the treatments of the C series at the low phosphorus level. In spite of the complication with ammonium toxicity, particularly in treatment M : P, these changes in the early rubidium effect among the four groups of treatments are in harmony with the corresponding general levels of internal phosphorus content (see Part I) at this time; in the M series at the high phosphorus level they are extremely high, while at the other extreme in the C series at the low phosphorus level internal phosphorus is so low as to constitute the main factor retarding growth.

Although phosphorus entry is retarded in treatments receiving rubidium,

these in general finally produce more tillers than corresponding treatments without it, a result which may or may not be similar to the effect on dry weight. As an instance of dissimilarity, treatment C : R has finally more tillers than C : O, but a much smaller dry weight; similarly with treatments C : KR and C : K. These four treatments are all clearly phosphorus limited (see Part I), those with rubidium more so than those without, and up to the third sample tillering behaviour corresponds with response in total dry weight. After this time tillering ceases in C : O and C : K, while it continues and may even be accelerated in C : R and C : KR. Under conditions of phosphorus limitation, then, the presence of rubidium superimposes a stimulatory effect on late tillering upon the effects ascribable to phosphorus.

At the other extreme, instances have been adduced of excess phosphorus having adverse effects and restricting final dry weight. Treatments M : PK and C : PK are presumably in this condition, their harvest dry weights being no greater than those of M : K and C : K at a much lower phosphorus level, and their general condition far worse. Similarly, treatment C : P finally suffers from excess phosphorus and yields a much lower dry weight than C : O. In each of these three comparisons tiller number is much greater at the higher phosphorus level. This disparity cannot be explained as an effect on tillering occurring before phosphorus content becomes deleterious, since in treatment M : PK deterioration is well advanced by sample 3 (July 10), but tiller number continues to rise rapidly during a further four weeks; a similar but more gradual late rise occurs in treatment C : PK. Moreover, Shih (1936) found that high sodium accompanied by excess phosphorus leads in general to successive cycles of new shoots and a prolongation of tillering far beyond the time it normally ceases. The evidence is strong therefore that the increase of tillering accompanying increase of phosphorus uptake does not necessarily cease even at levels of phosphorus which are injurious to the plant, but rather that ample—and possibly even excess—phosphorus is essential to the production of tiller numbers much exceeding those of normal well-nourished plants. The increased tillering of treatments receiving rubidium at the high phosphorus level, and its continuation late in life history, is not then a consequence of restricted phosphorus uptake, so that the increased final yield and tiller number consequent on rubidium application are not in these instances wholly or even largely due to the same cause.

Under conditions of both excess and restricted phosphorus therefore the presence of rubidium is a factor tending at least finally to increased tiller production, and the extent to which this occurs is dependent on the internal phosphorus supply, and presumably of course nitrogen supply as well. The fact that rubidium addition to treatments M : PK and X : P, with very high phosphorus contents, results in increased tillering from the beginning seems to indicate that this effect of rubidium is operative throughout development and is not confined merely to later stages; while treatment C : P results in as high a phosphorus content as does X : P, rubidium addition here exerts considerably greater restriction than in X : PR, accounting for the somewhat

reduced tillering in early stages of C : PR relative to C : P. The extreme length to which the stimulation of tillering by rubidium is carried under favourable conditions is well illustrated by the description in Part I of plants grown at the same high phosphorus level, but at a higher rubidium level than the present; these finally produced considerably more than a hundred tillers each, some five times the normal maximum number attained under similar cultural conditions by a well-nourished barley plant.

This effect of rubidium is therefore one aspect of the general toxicity postulated in the first part of the paper. In this respect rubidium appears to be similar to sodium, which as Shih (1936) found, tends also towards excessive and prolonged tiller production at low potassium and high phosphorus levels. The maximum number observed among his treatments, however, was not more than 30 per plant, even though the external sodium concentration was taken to many times the equivalent of the highest rubidium level used here. In this respect therefore rubidium is much more active than sodium. The mechanism underlying this effect of rubidium and sodium is entirely a matter for speculation.

The only instance in the data of a reduction in final tiller number due to rubidium is provided by the comparison between treatments M : K and M : KR. The reason for this is not clear, but there is evidence that rubidium exerts some restriction on phosphorus entry in early stages; thus at sample 1 the phosphorus contents of the leaves from the two treatments are 0.73 per cent. and 0.56 per cent. respectively. Since treatment M : K is already limited as regards tiller production by its phosphorus level (comparison between M : K and M : PK), this may account for the early difference in the rubidium effect at the two phosphorus levels; but by sample 3 the difference in phosphorus content has disappeared or is even reversed in sign. A reduction in the late tillering of treatment M : KR relative to M : K may have been brought about by the fact that the former ripened earlier than the latter, an unusual effect of treatment with rubidium. Finally, the discrepancy may not be so great as is suggested by the data, since evidently (Fig. 4) the three-pot sample from treatment M : KR on the third sampling occasion was very aberrant as regards tiller number, leaving the remaining five pots with considerably fewer tillers than the previous average for the treatment. Selection, if any, in treatment M : K at the same time was in the opposite direction. Thus the subsequent observed difference between these treatments was exaggerated, and possibly by the time of harvest tiller number in M : KR should have been at least as high as in M : K.

*Root : top ratio.* The percentage roots, or ratio of roots to tops, is in the main a reflection of the proportion of the total assimilate translocated to the root. A high carbohydrate plant usually has a high ratio (e.g. nitrogen deficiency in barley, &c.), and a low carbohydrate plant a low ratio, as frequently in potassium deficiency under conditions of high sodium supply. It therefore appears likely that the higher the carbohydrate level of the plant the greater is the proportion of the assimilate normally translocated to the

root.<sup>1</sup> The root : top ratio may be expected therefore to be positively correlated with assimilation rate. The correlation coefficient derived from the eighteen treatments of the present experiment, excluding the aberrant M : O and M : P, between the value of the ratio at sample 3 and the mean net assimilation rate from samples 1 to 3 (i.e. over the preceding month) is, however, quite low: +0.241; and a similar coefficient derived from the data collected by Shih (1936) from twenty-two treatments is also below significance level: +0.373. Examination of the data of both experiments reveals that the inclusion of tiller number as a third variate may improve the relation. For this purpose tiller numbers at the third sample have been used, and the total and partial coefficients of the three pairs of variates are given in Table VI, both from the present data and those of Shih.

TABLE VI

*Correlation Coefficients between Root : Top Ratio at Sample 3 (R), mean Net Assimilation Rate from Sample 1-3 (A) and Tiller Number at Sample 3 (T)*

|                  | Present data. | 5%    | 1%    | Shih's data. | 5%    | 1%    |
|------------------|---------------|-------|-------|--------------|-------|-------|
| $r_{RA}$         | +0.241        | 0.468 | 0.590 | +0.373       | 0.423 | 0.537 |
| $r_{RT}$         | -0.663        |       |       | -0.774       |       |       |
| $r_{AT}$         | +0.374        |       |       | +0.015       |       |       |
|                  |               |       |       |              | 2%    |       |
| $r_{RA \cdot T}$ | +0.703        | —     | 0.606 | +0.607       | 0.503 | 0.549 |
| $r_{RT \cdot A}$ | -0.836        |       |       | -0.840       |       |       |
| $r_{AT \cdot R}$ | +0.734        |       |       | +0.516       |       |       |
| $R_{R \cdot AT}$ | 0.847         | —     | —     | 0.864        | —     | —     |
| $R_{T \cdot AR}$ | 0.861         | —     | —     | 0.840        | —     | —     |

The two sets of data are in close agreement. Of the three total correlations, that between tiller number and the root : top ratio alone is significant, and is negative, but all three partial coefficients are highly significant, those with assimilation rate being positive. If we regard the root : top ratio as dependent on assimilation rate and tillering, over 70 per cent. of the total variance between treatments may be accounted for, and similarly if we regard tillering as the dependent variate.

It appears likely then that there is some measure of competition between the roots and young tillers for the available assimilate. While the data do not help to decide which of these organs has priority, it is reasonable to suppose that tillering rate is determined primarily by the phosphorus and nitrogen available for protein synthesis (cf. Richards and Templeman, 1936) or by hormones, and not by carbohydrate supply. The new meristems draw for some time on the carbohydrate produced by older tillers, resulting in reduced translocation to the roots. On this view, the large effect of phosphorus supply on the root : top ratio in the present experiment is not a simple one, but in

<sup>1</sup> Blackman and Templeman (1940) concluded that in grasses at low light intensity, and hence at low carbohydrate levels, leaves are produced at the expense of roots.

some treatments may be influenced predominantly by deviations in assimilation rate and in others by unusually large concomitant deviations in tillering rate. Again, the very high ratio frequently found under nitrogen deficiency, cited earlier, and the low ratio under potassium deficiency in high sodium nutrients will not be reflections simply of the relative amount of assimilate accruing to the plants, but also of the much reduced tillering rate found with nitrogen deficiency and the high rate characteristic of that type of potassium deficiency. The distinction is between existing and fully assimilating tillers having to provide only for their own needs and those of their roots, or having to meet also the heavy carbohydrate demands of new non-assimilating aerial organs; in either event the aerial parts of the plant appear to be more favourably placed than the roots to meet their requirements, which are considerably affected by the rate of tillering.

In the above analysis net assimilation rate is not perhaps a very good measure of the available assimilate, since differing treatments may result in different ratios of leaf area to total dry weight. Relative growth rate may be more satisfactory from this point of view, since it measures the net gain to the plant in terms of its weight. The replacement of net assimilation rate by relative growth rate in the analysis of the present data leads to no important change in the result, the partial coefficients remaining highly significant and of the same sign.

*Water content and death-rate of tops.* The complexity of the water content relationships between treatments, particularly at low potassium levels, has been demonstrated in a previous communication (Richards and Shih, 1940), and it may be recalled that significant positive correlations were there shown to exist between phosphorus and water contents. Potassium content was not shown to exert any consistent effect; sodium content on the contrary was closely related to the internal water level, and calcium content also appeared to exert an appreciable effect. The last two elements were positively correlated with water content when effects of varying contents of other elements were eliminated.

In the present experiment the raising of the external and internal phosphorus levels leads to increased succulence, a result apparently in agreement with the previous findings. While it is impossible without comprehensive chemical analyses to determine the specific effect of rubidium on water content, whether it behaves like potassium or sodium, or even differs from both and is inversely related, it is noteworthy that the addition of a comparatively small amount of the element to the nutrient almost invariably results in a considerable reduction in succulence. It appears then that in general, wherever the addition of phosphorus leads to increased succulence the addition of rubidium leads to a decrease. Application of both elements together results in a water content intermediate between those resulting from addition of the two individually. This relation is in accord with the hypothesis that some of the main effects of rubidium on the plant result from the restriction of phosphorus entry, but it is not suggested that the sole effect of rubidium on succulence is to be found

here. There must be a specific effect of the element itself, and if as is probable it affects composition in any other manner, these modifications will likewise be reflected in water content. There are moreover important exceptions to the generally opposed behaviour of rubidium and phosphorus contents, for at sample 3 treatment M : PKR has at least as high a phosphorus content as treatment M : PK (see Part I), while at the same time it has a considerably lower water content. This is not true of the earlier leaf samples from these treatments, and the result is probably associated with the rapid deterioration in treatment M : PK, an aspect to be referred to more fully shortly.

Attention has been drawn to the fact that variation in water content due to treatment is generally greater in 'stems' than in leaves, and in series M than in C, and it was noted that correlated with this difference the mean water content is lowest in leaves of C, higher in those of M, and highest in 'stems' of M. It might be objected that this result is to be expected so long as water content is measured in terms of dry weight, since the ratio of weight of water to weight of dry matter is greater than unity, i.e. a given change in dry weight will cause a much larger change in estimated water content if this is high than if it is low. That this explanation does not account for the result is clear from Table VII, in which are recorded the comparable results estimating water content in terms of fresh weight, thus giving a large bias in the opposite direction. At each sampling time are tabulated the mean water contents of eight treatments together with the variance due to treatment among those eight.

TABLE VII

*Correlation between Mean Water Content (per cent. fresh weight) of eight Treatments and Treatment Variance*

|            | Sample 1. |           | Sample 2. |           | Sample 3. |           |
|------------|-----------|-----------|-----------|-----------|-----------|-----------|
|            | Mean.     | Variance. | Mean.     | Variance. | Mean.     | Variance. |
| C, leaves  | 82.5      | 2.6       | 83.6      | 4.9       | 81.4      | 12.9      |
| M, leaves  | 83.8      | 9.6       | 85.1      | 23.5      | 83.1      | 35.6      |
| C, 'stems' | 86.3      | 17.9      | 87.3      | 39.7      | 83.8      | 52.6      |
| M, 'stems' | 88.8      | 18.6      | 88.6      | 18.2      | 86.2      | 72.4      |

It is apparent that the same general correlation holds at every sample in spite of the bias due to the method of calculation, a single aberrant result being found in the 'stems' of series M at sample 2. The relationship is presumably controlled by carbohydrate level, working through general differences of thickness of cell-walls between the groups, in the manner postulated by Richards and Shih (1940). A group with thick cell-walls will not only have a low average water content, but will also be resistant to modification of water content by internal factors. A similar correlation is not found in time. The mean water content is maximal at sample 2, corresponding with a minimal carbohydrate level occurring normally at about this stage, while the variation due to treatment continues to rise rapidly between samples 2 and 3. The

explanation of this may be in part that variation in internal salt content among the treatments increases with time owing to the content falling more rapidly in treatments producing a high growth rate than in those producing a low rate, but a more powerful factor is presumably that the normal carbohydrate accumulation in the later vegetative phase is much reduced under some treatments, particularly those with a very low assimilation rate. In consequence treatments tending towards maximal succulence at earlier samples do not decline between samples 2 and 3 to the same extent as treatments with a higher assimilation rate and lower water content at earlier phases. A similar difference was found by Richards and Shih (1940) between treatments at a very low potassium level and those at a high.

A further inverse relation between effects of phosphorus and rubidium is found in the amount of dead matter appearing during the sampling period, and in the percentage dead tops (or ratio of dead to living tops). With the possible exception of treatments at the low potassium level of series M, the data from which are necessarily uncertain, phosphorus in the absence of rubidium always increases both the absolute and relative amounts of dead matter. The addition of rubidium consistently reduces the rate of death at all tested levels of phosphorus and potassium. Potassium in the absence of rubidium also reduces the rate at both phosphorus levels, though its effect is never so great as is that of rubidium.

It is unlikely therefore that the large rubidium effect is to be explained simply as a partial substitution of the heavier element for potassium in nutrition. If at the high phosphorus level phosphorus toxicity is one of the major factors operative in the experiment, the rubidium effect here may be intelligible, although the same hypothesis will scarcely account for the considerable beneficial effect at low phosphorus levels in the C series, particularly when rubidium is applied to treatment C : K. For here phosphorus is limiting growth to at least as great an extent as is potassium, and the addition of rubidium (as also to treatment C : O) leads to no great modification of the internal phosphorus content, already very low, but instead to a much reduced growth rate throughout life and the exaggeration of phosphorus deficiency symptoms. The fairly large values of percentage dead tops in treatments C : K and C : O cannot then be accounted for in terms of excess phosphorus, although the approximate doubling of these values in treatments C : PK and C : P presumably must be. As was shown in Part I, these two latter treatments finally produce plants in a much worse general condition than do C : K and C : O, though in early stages an opposite relationship holds. At low potassium levels then the elimination of the possibility of excess phosphorus accumulating in the plant results in a considerable advantage, yet there are left other important causes of disorganization. To what extent the residual deleterious effects of potassium deficiency are ascribable to excessive accumulations of elements other than phosphorus is unknown, but the variability of the symptoms of deficiency in nutrient solutions of different compositions suggests that it may be considerable. While the greatly beneficial effect of



rubidium on leaf longevity in these treatments (C : K v. C : KR and C : O v. C : R) may be related to some specific residual potassium deficiency effect, there may also be a general improvement arising from the considerable restriction of growth here by rubidium. As a consequence of reduced growth in treatments C : KR and C : R, presumably because of restriction of phosphorus intake, the internal potassium content may be increased relative to treatments C : R and C : O,<sup>1</sup> leading to less disorganization. Opposed to any such effect is the presumption that in the presence of rubidium phosphorus supply is much more rigorously limiting growth, and in simple phosphorus deficiency leaf longevity is reduced. In the absence of concrete knowledge of the residual potassium deficiency effects further speculation concerning the exact rubidium effect here is unprofitable; but the elucidation of the problem may clearly lead to results important for the understanding of the effects of potassium in metabolism.

Among the various nutrient treatments of the experiment there appear to be fairly close correlations between the three variables net assimilation rate, water content, and the amount of dead tops produced. The particular values of the variables used to determine correlation coefficients are largely arbitrary, hence only general trends may be distinguished. If among the twenty treatments of the experiment the ratio of dead to living tops at the third sample be correlated with water content, at the same time of leaves or 'stems', and with the mean net assimilation rate over the preceding four weeks, the coefficients given in Table VIII are obtained.

TABLE VIII

*Correlation Coefficients between Net Assimilation Rate (A), Leaf (L) or 'Stem' (S) Water Content, and Ratio of dead to living Tops (D)*

| Variables. | Coefficient. | Variables. | Coefficient. |           |
|------------|--------------|------------|--------------|-----------|
| AL         | -0.55        | AS         | -0.51        | 5% = 0.44 |
| AD         | -0.65        | AD         | -0.65        | 1% = 0.56 |
| LD         | +0.49        | SD         | +0.64        |           |

All coefficients are significant, three of them very highly so. The series M and C are inconsistent in assessing the relative importance of the relationships, and if they are treated individually an extremely high negative coefficient may be obtained from series M between assimilation rate and dead matter, and a similarly high positive coefficient from series C between water content and dead matter. Assimilation rate and water content appear to be correlated to about the same extent in both series, and negatively; the relationship is

<sup>1</sup> This may well be so in spite of the observation of Collander (1941) that addition of rubidium depresses the absorption of potassium and vice versa, and that as regards absorption plants are unable to 'discriminate' between the two elements. For Collander's experiments were concerned solely with potassium in relatively high concentration, whereas in the present experiment the highest concentration even of the two elements together is very low, much too low for successful growth. Under these circumstances it is likely that nearly the whole presented quantity of both elements was eventually absorbed.

presumably largely a reflection of the effect of carbohydrate level on succulence discussed above. The positive correlations between death-rate and water content and the negative correlations between death-rate and assimilation rate probably indicate that the relationships in the main are due to the toxic effects studied in the experiment. Toxicity leads firstly to reduction in assimilation rate; secondly, through the resulting reduction in carbohydrate level, and possibly also through disruptive processes in the cell affecting osmotic pressure and hydration, to increased succulence, though this effect may be partly annulled by drying up with the onset of death; and finally to premature death.

An outstanding example of such a history is found in treatment M : PK, in which adverse symptoms appeared suddenly between samples 2 and 3 and developed rapidly. The estimated net assimilation rate during this period was 60 per cent. lower than during the previous fortnight. In all other treatments from the M series the water content of both leaves and 'stems' fell between samples 2 and 3, in most very considerably, as is normal at this stage of life history; in M : PK, however, there was actually an increase over the same period. At the same time, the leaves began to die off rapidly, starting in a highly characteristic manner with those of medium age; shortly after sample 3 the plants were in a sorry condition with very little leaf tissue still alive and assimilating.

*Ear and grain data.* Statistical average effects of rubidium in the ear and grain data are not in general as great as its interaction effects with phosphorus and potassium, and particularly with phosphorus in the C series; the observed effects have every appearance of being mainly indirect. Rubidium mean effects in series C are generally low, and may be positive or negative; quantitatively they are somewhat similar to main effects of phosphorus, which again are not usually great and may be negative, i.e. both rubidium and phosphorus on the average may be detrimental to grain production. Their interaction effects in this series are on the contrary always very large and positive, so that either element in the presence of the other is highly beneficial. The ear and grain data then are in agreement with the conclusions reached in Part I, that while the predominant effect of rubidium in the C series is to reduce the uptake of phosphorus, neither of the phosphorus levels used is well balanced physiologically with either of the potassium levels, and for maximum efficiency both potassium levels require a phosphorus level intermediate between those employed in the experiment; the internal phosphorus content is nearest the optimum in treatments given rubidium at the high phosphorus level.

In the ear and grain data of series M effects of rubidium, both average and interaction, are not in general large, and cannot be interpreted so easily in terms of phosphorus content as can those from series C; a similar situation in the dry weight data was discussed in Part I, and the data now under consideration appear to throw no further light on the question. Treatment with rubidium, however, is unlikely to have produced any large results in the harvest data which are not implicit in the effects of the element during the

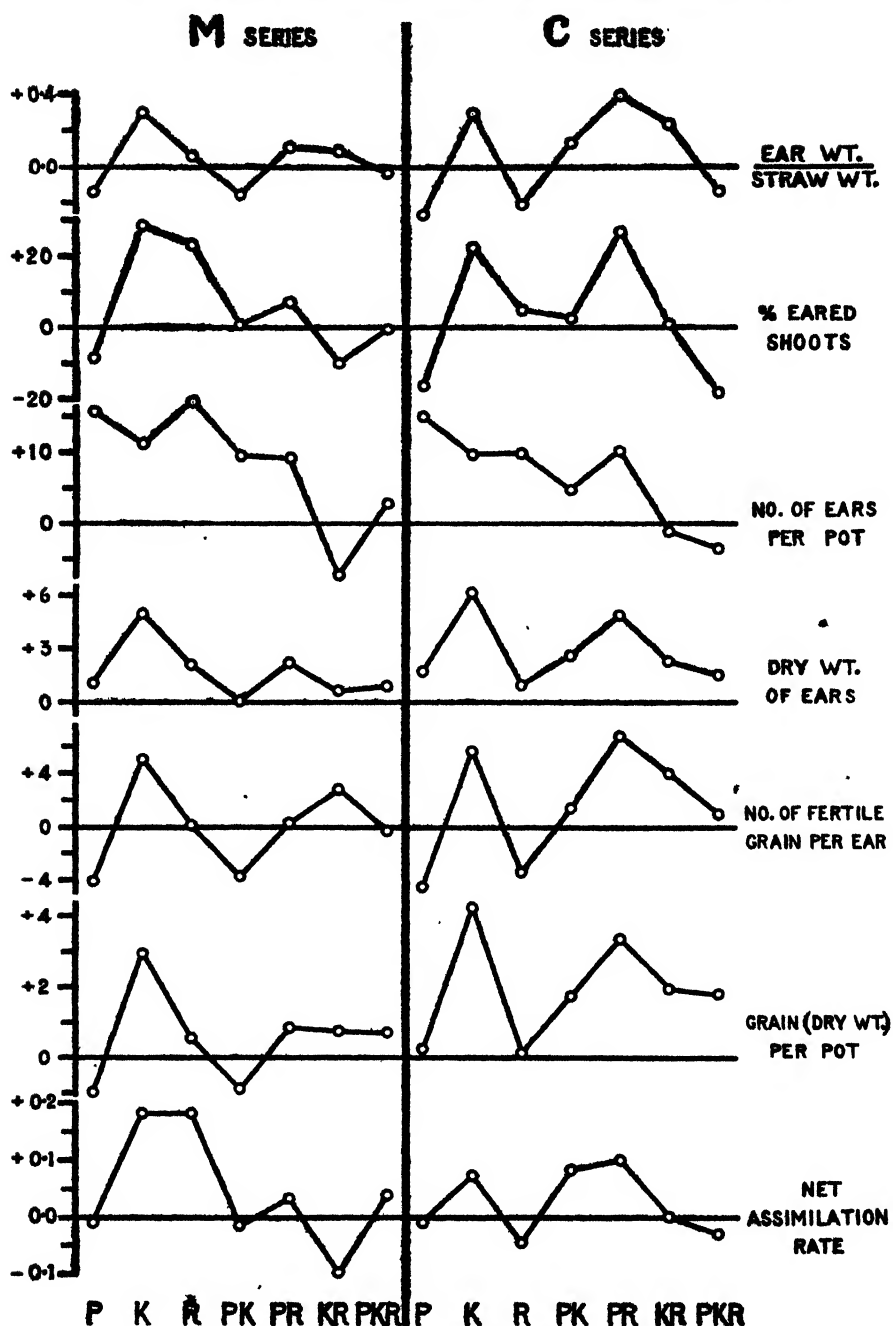


FIG. 7. Calculated statistical effects from harvest data and net assimilation rate of the M and C series, arranged to show correlations. Letters at the bottom of the diagram indicate the main and interaction effects of the three nutrient elements.

purely vegetative phases, already considered. Thus the statistical effects calculated from the mean net assimilation rates from samples 1 to 3 are included in Fig. 7, and it will be seen at once that there are close similarities between these effects and those from some of the ear and grain data collected at least ten weeks later. In series M the assimilation rate effects are remarkably similar to those of the percentage of shoots bearing ears; in series C too the calculated effects from these two variates are generally similar, though here the assimilation rate effects bear a closer resemblance to those derived from either the ear : straw ratio or the number of fertile grain per ear.

Turning to the primary data themselves, percentage eared shoots may be correlated with mean net assimilation rate, using the data derived from all nineteen treatments which produced ears in 1939. The coefficient reaches the high value of  $+0.797$ , the 1 per cent. point being  $0.575$ . The relationship is shown in Fig. 8. The diagram indicates also the regression line of percentage eared shoots on assimilation rate, and the mean effects of the nutrient variables: basal treatment (M, C, and X), phosphorus, potassium, and rubidium. The other lines show the individual single effects consequent on rubidium addition. A point for treatment M : P has been inserted using a value of percentage eared shoots found in the previous season. If the values shown for treatment M : P be included in the correlation, the coefficient rises to  $+0.849$ ; while if both treatments M : P and M : O are omitted, since they are in many respects aberrant, the coefficient is unchanged at  $+0.797$ , but the regression line is somewhat steeper. From the evidence presented in the diagram there is little reason to assume that variation in any of the nutrient variables has a large specific effect on the slope of the regression line.

Exactly similar considerations apply to the relationship between the ear : straw ratio and net assimilation rate. The correlation coefficient derived from the nineteen treatments producing ears is  $+0.626$  (1 per cent. =  $0.575$ ). As might perhaps be expected, treatment M : O is again aberrant; assimilation rate was observed only during the period when it was excessively low in this treatment and the plants were rapidly losing weight relative to those from other treatments. Between sample 3 and harvest a considerable improvement in dry weight occurred relative to the other treatments, and this was presumably accompanied by a relative—if not an absolute—increase in assimilation rate. If then this treatment be omitted from the correlation, the coefficient rises to  $+0.742$ . An analysis for these variates similar to that of Fig. 8 again gives little indication of specific effects of any of the nutrient variables on the slope of the regression line on assimilation rate. It is interesting to note that the correlation coefficient for these two variates derived from the twenty-two treatments of Shih's experiment (Shih, 1936; cf. Richards and Shih, 1940) is almost the same as that from the present experiment, namely  $+0.752$ ; again in his data there is no indication of specific effects of the various nutrient variables.

The relationship between the number of fertile grain per ear and net assimilation rate may finally be examined. As before, and apparently for the

same reason, treatment M : O is aberrant, and will be omitted, leaving eighteen treatment pairs for correlation. The coefficient is rather lower, +0.685, though still highly significant (1 per cent. = 0.590). The relationship here to rubidium application differs from those in the two regressions

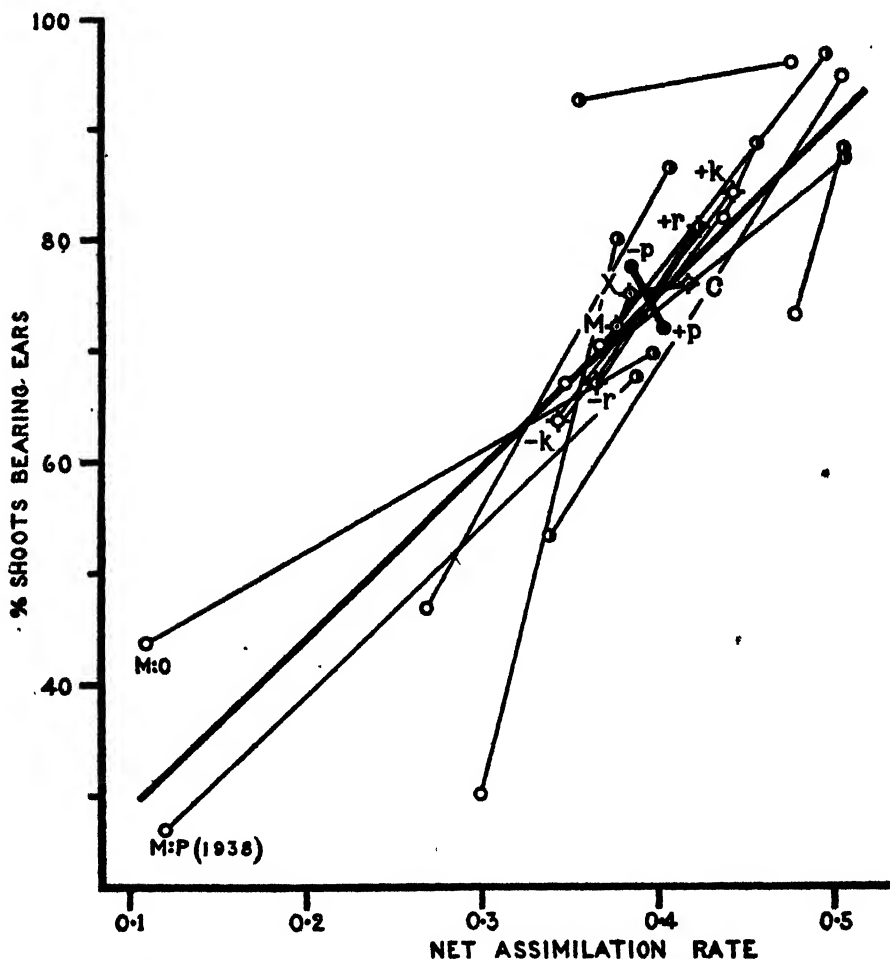


FIG. 8. Correlation diagram for all twenty treatments between percentage shoots bearing ears and mean net assimilation rate. The regression of percentage shoots on assimilation rate is indicated. Mean effects of the type of nutrient (M, X, and C), of phosphorus, potassium, and rubidium are shown, together with the ten individual rubidium effects.

already considered in that addition of the element to either treatment C : P or X : P *reduces* the number of fertile grain per ear while increasing assimilation rate; and the available information indicates that a similar inverse correlation exists between treatments M : P and M : PR and between M : O and M : R. The remaining rubidium comparisons behave normally, so that where the element decreases assimilation rate (C : O and C : K) it decreases

fertility per ear also, and vice versa ( $M : K$ ,  $M : PK$ ,  $X : PK$ , and  $C : PK$ ), and these changes may be large. The means with and without rubidium again lie close to the regression line, much nearer than the fiducial limits. The aberrant behaviour with rubidium addition to the four treatments mentioned is clearly not a specific effect of rubidium on fertility within the ear, since the other six treatments behave in a diametrically opposed manner; hence we are concerned with large interaction effects. In these particular four comparisons some factor other than assimilation rate is dominant.

Three of the treatments behaving aberrantly with rubidium addition are the three combinations of very low potassium with high phosphorus, the fourth being  $M : O$ , again with minimal potassium but with reduced phosphorus. These four treatments showed much the greatest responses to rubidium in tillering rate found in the experiment and, with the exception of  $X : PR$ , when given rubidium tillering continued at a fast rate until a very late final harvest date; as stated in Part I, many of the plants from treatment  $X : PR$  were practically dead at the time of harvesting, although the dry weight at this time was actually greater than in treatment  $X : PK$ . Again, as may be seen in Table V, the percentage eared shoots in all these treatments without rubidium were much the smallest of the experiment, while their responses to rubidium in percentage eared shoots were excessively large, generally larger than the corresponding responses to potassium. It is possible that such large responses in tillering rate, vegetative dry matter production, and earing in plants at an extremely low potassium level exhausted them in some manner, so that practically no fertile grain was produced.

Because of the correlations existing between certain of the ear and grain data and net assimilation rate, it might reasonably be suspected that this exhaustion is one of available carbohydrate, and indeed that the correlations themselves are due simply to the obvious need for carbohydrate of the reproductive organs and its supply by the leaves and stems. In view of the work of Watson and Norman (1939), and especially of Archbold (1942) on the relation of ear development to sugar metabolism and storage, so simple an hypothesis appears unlikely, for it is claimed that the ears themselves contribute largely to their own needs. In that event available carbohydrate in the vegetative organs might determine whether or not a tiller would produce an ear in the first instance, but presumably be of minor importance in determining the final weight reached by the ear and probably also fertility within the ear. It might, however, be maintained that between treatments, assimilation rate of the ears themselves is likely to be closely correlated with the assimilation rate of the vegetative organs observed many weeks earlier, thus leading to the correlations discussed above.

On the other hand, it is more than possible that assimilation rate and fertility are not causally related, and that both processes are dependent on some other more general physiological state. In the experiment, potassium level is everywhere low, and important statistical effects in both assimilation rate and fertility are ascribable to potassium itself. We are dealing everywhere with

conditions of deficiency of the element, and emphasis in the interpretation of the results has been placed on toxicity resulting from the accumulation of other ions under these conditions. It may very well be that both assimilation rate in the vegetative phase and fertility later are general measures of ionic balance within the plant.

At the same time it appears certain that potassium itself has an essential direct function in metabolism quite apart from any indirect toxicity effects; under varied nutrient conditions the latter may lead to diversity in both external and internal symptoms of potassium deficiency, but no arrangement of the other nutrient conditions will allow the development of a normal plant. It is possible of course that potassium plays a direct role in determining fertility within the ear. If so, this might account for the reduction in fertility accompanying increased assimilation rates occasioned by the addition of rubidium to the four treatments C : P, X : P, M : P, and M : O. Here the great increases in tillering rate and production of green leaves late in life history may use the available potassium within the plant, while the mere presence of rubidium may even reduce the actual uptake of potassium (cf. Collander, 1941). Evidence from the other treatments, however, is not entirely favourable to this hypothesis; in particular, the fifth treatment at the very low potassium level in the absence of rubidium, C : O, produced the largest final dry weight of the five, and, in spite of any internal dilution of potassium due to the greater growth, the number of fertile grains per ear was as high as in any treatment of the experiment, and much higher than in the other four low potassium treatments. A well-marked distinction again occurs between treatment C : O and the other four, for whereas all the latter showed renewed tillering very late in life history, C : O ripened normally and early; hence all available potassium in the plants from treatment C : O may have been translocated to the ears. It is impossible on the evidence to decide whether failure in the ears leads to a renewal of tillering, or vice versa, but it is clear that under potassium deficiency grain production and late tillering must be competitive processes, if not for carbohydrate, at least for potassium itself.

Finally, the data for the 1,000-grain weight have in some treatments necessarily been estimated from a very few grains, and here the figures tabulated are quite unreliable. Rubidium treatment appears generally to reduce grain size, though it is again likely that the effect is wholly indirect. Grain size is negatively correlated with both the number of fertile grain per ear and net assimilation rate, the latter coefficient alone reaching significance level; further analysis throws no more light on the relationship, as might be expected from the unreliable nature of some of the data. Very similar correlations may be extracted from the data of Shih (1936) provided that all observations at the highest potassium level are discarded, so that we are again dealing always with conditions of potassium deficiency. In these data elimination of effects of the variate number of fertile grain per ear from the correlation between grain size and net assimilation rate reverses the sign, but the positive coefficient

is again insignificant. It seems likely, however, that the relationships indicated are real, and that under conditions of potassium deficiency, usually with considerably reduced assimilation rates, grain size is inversely related to the number of grain produced in the ear, while for a constant number of grain per ear a direct relationship may be expected between size and assimilation rate.

#### SUMMARY

Further data are presented from a growth experiment with barley already described (Richards, 1941a). The experiment was designed to investigate some effects of rubidium application to plants deficient in potassium when grown at two phosphorus levels in two basically different types of solution: (1) high ammonium with low calcium, and (2) high calcium without ammonium; a solution of intermediate composition was also used at the high phosphorus level. The following are the main results.

1. Differences in net assimilation rate account for most of the observed differences in growth under the various treatments, and it is suggested that in the experiment internal phosphorus level is one of the dominant factors determining assimilation rate. Reduced rates are to be expected either when phosphorus is deficient or when present in excess.

2. Phosphorus level is also a dominant factor in determining the rate of tillering, and early effects of rubidium on tillering apparently have their origin in previous restrictive effects on phosphorus uptake; except where phosphorus is in excess, rubidium reduces early tillering. The final effect of rubidium, even at low phosphorus levels, is to increase the number of tillers, and the increase may be large; this is interpreted as a toxicity effect, and a resemblance between rubidium and sodium in this connexion is indicated.

3. Phosphorus has a large effect in reducing the root : top ratio. Correlations between the values of this ratio, net assimilation rate, and tiller number among the treatments support the hypothesis that competition occurs between the roots and young tillers for available assimilate, and hence that root size in any treatment is dependent on the amount of tillering induced by that treatment.

4. Water content in the experiment is increased by raising the phosphorus supply and reduced by application of rubidium; potassium supply exerts no consistent effect. Treatment effects are largest in groups of treatments with highest mean water contents, this being interpreted as an effect of carbohydrate level.

5. The ratio of dead to living tops is decreased by raising the potassium level, and much decreased by an equivalent application of rubidium; this is one of the outstanding effects of rubidium in the experiment. The toxicity of excess phosphorus and its reduction following rubidium addition is insufficient to account for the whole result. Correlations existing between net assimilation rate, water content, and the ratio of dead to living tops presumably arise largely from toxicity effects in the experiment; toxicity leads firstly to



reduction in assimilation rate, secondly to increased succulence, and finally to death of leaves and tillers.

6. The ear and grain data show large differences in the rubidium effects at the two levels of phosphorus. Effects of rubidium are probably indirect and consequent on previous effects during vegetative phases. Highly significant correlations exist between net assimilation rate during the vegetative period and many of the ear and grain data, and these correlations appear to be largely independent of the nutrient conditions causing the observed differences in assimilation rate. Certain possible causes of sterility within the ear are discussed, and the conclusion reached that grain production and unusually late tillering, such as frequently occur under potassium deficiency, are competitive processes: if not for carbohydrate, at least for potassium.

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# **A Comparison of the Effect on Plant Growth of Micro-elements and of Indole Acetic Acid and Colchicine**

BY

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**T**HE indispensability of micro-elements in plant growth has been clearly demonstrated by a number of workers, e.g. Steinberg (1935) and Stout and Arnon (1939). But up to the present, neither the cause of this indispensability nor the mechanism of the action is yet understood. Tang and Yao (1942) in this laboratory showed that manganese sulphate in adequate concentration was very effective in inducing curvature in *Avena*, thus throwing some light on the function of manganese. During the last three years we have paid much attention to this problem, and a series of experiments has been carried out dealing with the physiological role of manganese and other micro-elements in the growth and development of plants. The results of these investigations are here summarized briefly. In our experiments the effect of indole-3-acetic acid and of colchicine has usually been studied at the same time as that of the trace element.

## *The effect of manganese sulphate, indole-3-acetic acid, and colchicine on germination and early growth.*

Extensive work has been done on the effect of manganese sulphate, indole-3-acetic acid, and colchicine on the germination and early growth of crop plants. Seeds were soaked in sterilized water and placed in Petri dishes containing 5 c.c. of solutions of manganese sulphate, indole-3-acetic acid, and colchicine respectively with distilled water as control. After determining the percentage of germination, 100 plants were selected from each test solution and transferred into tall glass vessels containing 10 c.c. of fresh test solution for further growth observation. Using this technique, Loo (1942) found that, in concentration range of 1–50 mg. per litre, manganese exerted a favourable effect on the seed germination and early growth of the rice plant. Its presence increased the percentage of germination, the elongation of the shoot and especially of the root, and finally the amount of dry weight. Colchicine in the same concentrations also accelerated the growth of young rice plants. On the other hand, the growth of rice seedlings in the indole-3-acetic acid solution was inferior to that in solutions of manganese sulphate and colchicine. The growth was apparently abnormal and the percentage of germination small, although in low concentrations it was increased a little. Similar results have

been obtained by Loo and Tang (1944, in press) using corn, mung bean, and cabbage as test plants. The concentrations of the test solutions used ranged from  $10^{-2}$  to  $10^{-8}$  M. Manganese sulphate in concentrations less than  $10^{-3}$  M. always accelerated the germination rate and exerted some beneficial effect on the early growth. Indole-3-acetic acid in the same concentration range was found to be detrimental. To corn and cabbage indole-3-acetic acid in higher concentrations was distinctly growth-inhibiting. The effect of colchicine was somewhat similar to that of manganese except that in high concentrations ( $10^{-2}$  and  $10^{-3}$  M.) it was definitely toxic. The after effect of seed treatments on the germination and early growth of wheat and rice has also been studied. After soaking, the seeds were treated with test solutions whose concentration varied from 100 to 200 mg. per litre for periods of 12, 24, and 48 hours. The seeds were then washed carefully with redistilled water and set for germination and growth observations in the dark. In general, manganese sulphate in adequate concentrations exerted good after-effect on the germination and growth of the wheat plant, provided that the time of treatment did not exceed 24 hours. If time was prolonged or a solution of higher concentration used it resulted in a reduction of germination rate. The after-effect of colchicine was discernible in the rate of germination, though there was no significant influence on the early growth. On the other hand, the pre-treatment with indole-3-acetic acid always resulted in the inhibition of germination, even with short periods of treatment. It increased somewhat the dry weight of the root but definitely inhibited the growth of the shoot.

That the beneficial effect of manganese sulphate on germination and early growth was due to the cations has been proved by Tang Yü-Wei in this laboratory (unpublished data). Corn seeds were set for germination in solutions containing manganese sulphate, manganese chloride, and manganese nitrate respectively in different concentrations. The germination of the seeds was accelerated and the early growth of seedlings promoted. Pre-treatment of corn seeds with these solutions of manganese salts also resulted in growth promotion. The most effective concentration was found to be  $10^{-2}$  M. Among these three salts manganese nitrate was especially effective in promoting shoot growth.

#### *The effect of micro-elements other than manganese.*

The effect of micro-elements other than manganese on the seed germination and early growth of wheat plant has been studied by Tsui Chêng (unpublished data) in this laboratory. Among several substances used, zinc sulphate, copper sulphate, ferrous sulphate, nickel sulphate, strontium sulphate, cobalt chloride, boric acid, and molybdic acid in concentrations, varying from 0.01 to 1.0 mg. per litre, were found to be beneficial in the same manner as manganese sulphate. The effective range of concentration was naturally very variable; thus the upper limit of concentration for zinc sulphate, boric acid, and manganese sulphate was greater than 1.0 mg. per litre, while

that for copper sulphate was 0.01 mg. Higher concentrations accelerated the germination of seeds but usually retarded the rate of growth.

*The effect of manganese sulphate, indole-3-acetic acid, and colchicine on pollen germination and pollen-tube growth.*

The influence of manganese sulphate, indole-3-acetic acid, and colchicine on pollen germination and pollen-tube growth has been studied by Loo and Huang (1944) in this laboratory, using the pollen of several plants, including rice, wheat, barley, tobacco, tea, snapdragon, and several species of *Allium*. The pollen grains were germinated in hanging drops containing sugar plus the test substance, the concentration of which ranged from  $10^{-5}$  to  $10^{-10}$  M., with sugar solution as the control. At the end of several hours the percentage of germination was determined and the length of pollen tubes was measured with a micrometer. The results of these experiments showed that the rate of pollen germination was promoted by manganese sulphate, colchicine, and even indole-3-acetic acid, and that this effect increased with the increase of concentration. In every case the effect of manganese sulphate and of colchicine was superior to that of indole-3-acetic acid, which retarded the rate of germination of rice in higher concentrations. These conclusions held good for the elongation of pollen tubes. Another interesting outcome was the morphological responses of pollen tubes to the test solutions. Those in the manganese sulphate elongated rapidly and produced fine, slender, and straight tubes. The elongation of pollen tubes in the colchicine-medium was also good, but the tubes were usually coiled in circles or spirals; the membrane of pollen tubes was not smooth but rather zigzag in appearance. Pollen tubes in the indole-3-acetic acid medium were short and stunted with swellings at the tip and base; bursting of pollen tubes also occurred frequently in this medium. These abnormal growth features of pollen tubes were similar to those of rice roots (Loo, 1942) and root-hairs of cabbage seedlings (Tsui Chêng, 1943, in press) treated with indole-3-acetic acid.

*Histological responses of the stem of sunflower to manganese sulphate, indole-3-acetic acid, and colchicine.*

From the above account it is evident that the effect of manganese and colchicine on the germination of seeds, the early growth of seedlings, the pollen germination, and growth of pollen tubes was beneficial while that of indole-3-acetic acid was unfavourable. Although both indole-3-acetic acid and manganese sulphate can induce curvature when applied unilaterally on the decapitated coleoptile of oats, their mode of action seemed to be different. Manganese sulphate accelerated normal growth, for under its influence the rate of growth was increased. The presence of indole-3-acetic acid, however, usually caused hypertrophied and abnormal growth at the region of application, but retarded the growth of an organ or a plant as a whole. These statements were substantiated by the facts recently found by Tsui Chêng (unpublished data) in this laboratory. On application of indole-3-acetic acid,

colchicine, and manganese sulphate in lanolin paste to the cut surface of the decapitated stems of sunflower, Tsui found that the histological responses of sunflower stems to these three substances were quite different. Responses of sunflower stem to indole-3-acetic acid caused bud inhibition, the production of adventitious roots and the initiation of vigorous cell division, thus resulting in the formation of a large tumour and the development of horizontal cambium. Cells on the surface of application changed fundamentally both in shape and in position. Colchicine induced the swelling and enlargement of cells beneath the cut surface and the formation of a thin and smooth callus and of giant cells with many nuclei. In contrast with the cases of indole-3-acetic acid and colchicine the effect of manganese sulphate on the decapitated stem was insignificant, the histological responses being little different from the controls. The determination, however, of the number of axillary branches and their fresh weight showed that manganese sulphate promoted the growth of sunflower branches. The growth of the sunflower plant under the effect of manganese appeared normal and no noticeable histological change was observed.

*Effect of micro-elements, indole-3-acetic acid, colchicine, and some anaesthetics on the elongation of wheat coleoptiles.* \*

Evidence consistent with the above has also been obtained in experiments carried out on the elongation of wheat coleoptiles by Miss Lu Ting-Chih in this laboratory. Germinating seeds of wheat were transferred from Petri dishes to paper-lined beakers containing a series of dilutions ( $10^{-4}$ – $10^{-8}$  M.) of  $\text{MnSO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{CuSO}_4$ , and  $\text{H}_3\text{BO}_3$ , indole-3-acetic acid, colchicine, and chloral hydrate and formaldehyde, their root system being immersed in the solution. The length of coleoptiles was measured twice a day and the growth curves drawn by plotting length against time. All the experiments were made in the dark. Although this is not the place to enter into the details of the results, which will be published elsewhere, it should be pointed out that indole-3-acetic acid, in concentrations higher than  $10^{-7}$  M., inhibited the elongation of wheat coleoptiles but exerted no significant effect in lower concentrations, while the sulphates of zinc and manganese and boric acid gave favourable results in a wide range of concentrations. Copper sulphate, however, retarded the elongation at the beginning of growth and was very toxic in high concentrations. Formaldehyde and chloral hydrate in lower concentrations showed a little growth stimulation at first, but resulted later in an unchanged or lower degree of elongation. On the other hand, higher concentrations of these two substances suppressed the elongation of wheat coleoptiles at the very beginning, though the ultimate growth was somewhat similar to, or even slightly better than, the control. The same conclusions may be drawn for the effect of colchicine. By comparing the growth curves of coleoptile, the substances used in these experiments may be divided into three different groups. To the first group, which may tentatively be termed the essential elements for plant growth, belong the sulphates of manganese

and zinc and boric acid, which with a proper concentration accelerate the elongation of wheat coleoptiles. Formaldehyde, chloral hydrate, and colchicine belong to the second group which induces 'chemical stimulations'. Copper sulphate represents the third group which is toxic to plant growth. Indole-3-acetic acid in higher concentrations belongs to the third group, while in lower concentrations it belongs to the second. In the experimental conditions employed there was no indication of growth promotion by this acid.

*Relation of manganese, indole-3-acetic acid, and colchicine to other growth phenomena.*

Some experiments dealing with the effect of manganese, indole-3-acetic acid, and colchicine on the tuberization and the flowering of the potato have been carried out by Ni Tsin-Shan in this laboratory. Preliminary observations indicated that the effect of manganese was superior to that of indole-3-acetic acid for tuberization and production of wet weight, while for flowering the reverse seemed to be true. Whether there is any relation between the vegetative growth and flowering remains to be determined. Further experiments with this in view are in progress.

*The effect of micro-elements, indole-3-acetic acid, and colchicine on the metabolic activities of plants.*

Data obtained in this laboratory show that manganese and other micro-elements in general promote the growth of plants while indole-3-acetic acid does not; colchicine in lower concentrations usually exerts a good effect on growth but inhibits in higher concentrations. The question arises as to which of the two can be called 'growth-promoting substance'. A substance inducing *Avena* curvature may not necessarily be a growth-promoting material. In our view a substance which does not bring about a higher percentage of germination, more, longer, and thicker stems and branches, larger leaves, more vigorous roots, and, in general, a greater dry weight cannot be claimed as a growth-promoting one.

Though manganese sulphate can induce curvature in the *Avena* test it is apparently not a hormone. In all probability manganese and other micro-elements only act as catalysts in accelerating various biological reactions. In spite of the enormous amount of work on the influence of minor elements on plant growth, work dealing with their effect on the metabolic activities are scanty. Some experiments in this direction are now in progress in this laboratory. The first series aims to ascertain the effect of micro-elements and of indole-3-acetic acid and colchicine on the synthesis of dry matter by the leaves of *Ricinus communis* and *Helianthus annuus*, using the half-leaf method. The object of the second is to study the effect of these substances on the carbohydrate and nitrogen metabolism of the leaves of kidney-bean, and then the effect on the digestion of starch in germinating seeds.

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**Physiological Studies in Plant Nutrition**  
**XIV. Sugar Metabolism in the Barley Stem in Relation to Ear Development**

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**With seven Figures in the Text**

**INTRODUCTION**

**I**T is now recognized that, apart from the leaves, the stems and ears of cereal plants contribute by assimilation to the carbohydrate of the ear. Stems thus fulfil a triple function, as translocatory, as storage, and as assimilating organs. Their function as storage organs has already been studied in some detail by Archbold and Mukerjee (1942) and by Archbold (1942). They found that rate of loss of stored sugar from the stem is unaffected by defoliation and by shading of the ear, treatments which were designed to create a demand by the ear for stored carbohydrate; the rate of loss from the stem depends rather on the maximum concentration reached, and sugar depletion appears to be the result not of upward translocation but of the senescence of the stem. The sugar accumulating in the stem prior to flowering originates in the leaves rather than by autonomous assimilation *in situ*, and this accumulation can be virtually prevented by early defoliation, but is only slightly affected by shading the ear. Finally the distribution of the leaf-assimilate between stem and ear is determined by conditions other than carbohydrate supply, as for example nitrogen supply. As regards the assimilatory function of the stem, its maximum contribution to the carbohydrate supply of the ear has been estimated to reach 30 to 40 per cent. of the final dry weight of the ear, but this estimate involves a number of assumptions introducing errors of unknown magnitude and may be too high.

Confirmation of the above conclusions has now been sought by investigating the effect on the normal drift of sugar of shading the stem, the leaves and ears remaining meanwhile exposed to light; and by a second experiment in which ears were removed with and without simultaneous defoliation. The bearing of the results on the estimate of the assimilatory capacity of the stem has also been considered, but as will appear in the sequel, a considerable degree of uncertainty in this respect still remains.



## EXPERIMENTAL PROCEDURE AND METHODS OF ANALYSIS

Barley (Spratt Archer) was grown in 10-in. pots in soil in the manner described for the earlier experiments (Archbold, 1942). Seeds were sown on May 19, 1942, and plants were finally thinned to six per pot. As before, plants were grown under conditions of partial nitrogen deficiency. To the stem-shading experiment 48 pots were assigned, stems being shaded in 8 pots on each of three occasions, 50, 66, and 80 days after sowing respectively. Duplicate samples of one pot were collected from each of the treated groups and from appropriate controls at 7, 17, and 35 days after shading, and a final collection made when the grain was fully ripe, 139 days after sowing. To the ear removal and defoliation experiment, 32 pots were assigned. These treatments were given 66 days after sowing to each of 8 pots, leaving 8 untreated controls. Duplicate one-pot samples were collected 1, 2, 15, and 49 days after treatment, the final collection being at the time of the maximum dry-weight of ear, 115 days from sowing.

In both experiments only the four largest tillers of each of the six plants in a pot were shaded or had their ears removed, but in the case of defoliation all leaves were removed. All analyses were carried out on samples from the four largest tillers. With one exception where ears were too small for convenient excision, samples were divided into leaves, flag-leaf sheaths, stems (including sheaths other than that of the flag leaf), and ears. After weighing, the samples were cut up and mixed, and aliquots taken for dry-weight determinations, and for preservation in alcohol for sugar and residual-dry-weight determinations. Total sugar estimates include fructosans, and residual dry weight refers to material insoluble in alcohol and cold water. Details of the technique have already been described (Archbold, 1938, 1942).

## EFFECT OF SHADING THE STEM

Shading of the stem was effected by covering with strips of copper foil, lacquered to prevent excessive weather action and possible copper poisoning of the soil. Each strip had a central half-cylinder to enclose the stem, and two flanges of unequal width ( $\frac{1}{2}$  in. and  $\frac{3}{8}$  in.), and was initially 12 in. in length. The strips were cut with scissors into suitable lengths for covering the separate internodes, the spaces left for the leaves being as narrow as possible. Sections were used in pairs to cover each part of the stem, the wider flange of each strip of the pair being folded over the narrower flange of the other, thus completely enclosing the stem. The covered stems were supported by a rigid wire frame, consisting of two concentric rings attached to three legs driven into the soil. The stems were arranged between the two rings and suitably tied to prevent movement. The shading technique is shown diagrammatically in Fig. 1. Daily examinations were made of the plants, and additional sections added to cover newly grown portions of the stem.

The first occasion of shading, 50 days after sowing, preceded the appearance of awns by about a fortnight, and ears were then all less than 2 cm. in length.

Stems were therefore covered during most of the period of ear development and part of that of rapid stem elongation, when sugar is normally accumulating. The third occasion, 80 days from sowing, was arranged to coincide with

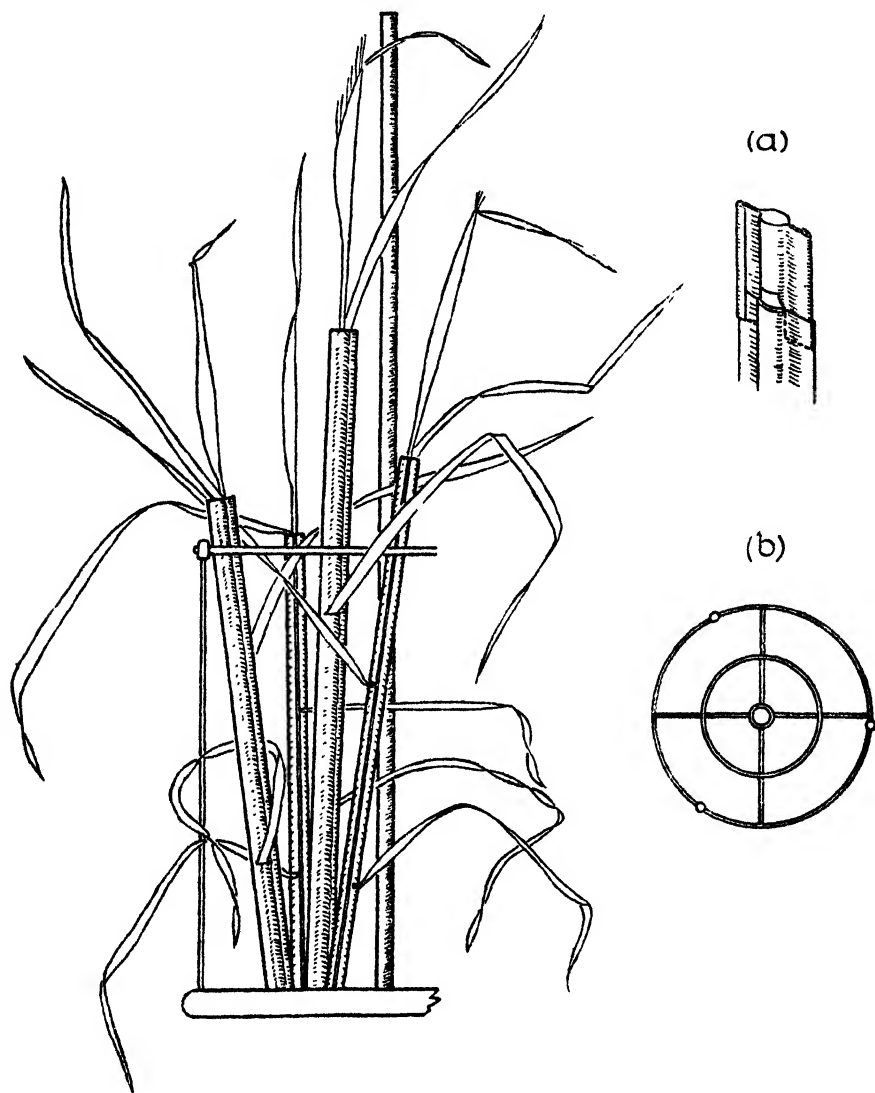


FIG. 1. Diagram illustrating the method of shading the stem, showing one plant only. (a) Piece of copper strip showing method of application and slit left for leaf. (b) Plan of wire frame to which shaded tillers were tied. See Text.

the maximum sugar content of the stem, and here ears had already emerged when treatment was applied. The second treatment occasion, 66 days from sowing, provided an intermediate stage between these two extremes. After

the early shading rapid stem extension continued for about 10 days in both treated and control plants, followed by a slow continued extension for a further fortnight, mainly in the younger two of the four shaded tillers. After the shading of intermediate date there was only slow extension of the stem in treated plants, and after the date of the last shading no further stem growth occurred.

Shading of the stem delayed emergence of the ear, restricted water loss from the stem itself, and increased its extension growth. Thus 15 days after covering the stems on the first occasion awns were showing on 24 stems in 6 control pots, but in 6 corresponding pots with shaded stems only one had appeared; 5 days later the numbers were 93 in the control and 41 in the treated. With regard to water content the values at the final collection were 170 per cent. of the dry weight for shaded stems and 106 per cent. for controls. Measurements showing the effect of stem shading on tiller height are given in Table I.

TABLE I

*Sums of Heights to Flag-leaf Auricle, and of the Lengths of the Top Stem Internodes of the Main Axis and the first Tillers of Each of six Barley Plants from a single Pot*

*s* = control; *S* = shaded. Sowing date, 19.5.42

| Time of shading (days after sowing)       | 50         |          | 66         |          | 80         |          |
|---|------------|----------|------------|----------|------------|----------|
|   | <i>s</i>   | <i>S</i> | <i>s</i>   | <i>S</i> | <i>s</i>   | <i>S</i> |
| <i>Heights to flag-leaf auricle (cm.)</i> |            |          |            |          |            |          |
|   | 733.5      | 812.5    | 679.0      | 756.5    | 758.5      | 726.5    |
|   | 706.5      | 715.0    | 721.5      | 807.5    | 708.0      | 724.0    |
|   | 717.0      | 784.0    | 728.5      | 730.0    | 681.5      | 717.0    |
|   | 725.0      | 767.0    | 696.0      | 803.5    | 727.0      | 681.5    |
| Treatment totals                          | 2882.0     | 3078.5   | 2825.0     | 3097.5   | 2875.0     | 2849.0   |
| Differences between totals                | 196.5 ± 84 |          | 272.5 ± 84 |          | -26.0 ± 84 |          |
| <i>Lengths of top internodes (cm.)</i>    |            |          |            |          |            |          |
|   | 148.0      | 176.0    | 151.5      | 225.0    | 168.0      | 164.0    |
|   | 160.5      | 216.5    | 157.0      | 223.5    | 147.0      | 165.0    |
|   | 147.0      | 195.5    | 160.0      | 161.5    | 154.5      | 148.5    |
|   | 157.5      | 174.0    | 147.5      | 233.0    | 154.5      | 153.5    |
| Treatment totals                          | 613.0      | 762.0    | 616.0      | 843.0    | 624.0      | 631.0    |
| Differences between totals                | 149.0 ± 48 |          | 227.0 ± 48 |          | 7.0 ± 48   |          |

Statistical analyses of the data showed that for the top internode the effect of shading and the interaction with time of application were both significant; while for the heights to the flag-leaf auricle the shading effect was significant, the interaction only just failing to reach the 5 per cent. level. Extension growth is therefore increased when stems are shaded early, but is unaffected when treatment is given after normal growth is complete. The greater part of the increase is in the top internode which is the last to extend, the increase

here accounting for 75 per cent. of the total with stems shaded 50 days from sowing, and 83 per cent. for shading 66 days from sowing.

The analytical data consist of duplicate determinations of dry weight, residual dry weight and total sugar in leaves, flag-leaf sheaths,<sup>1</sup> stems including sheaths other than that of the flag-leaf)<sup>1</sup> and ears for each of the four collections, making eight observations for each constituent for each of the three occasions. Of the three factors in this  $2 \times 3 \times 4$  factorial arrangement the time of application (*O*) and the interval between shading and collection (*A*) are arbitrarily chosen and much of the numerical difference attributable to them arises from the fact that nearly two-thirds of the growth cycle is covered by the collection dates. These 'factors' are therefore only of interest in so far as they modify the effect of the third factor, stem shading (*S*). A summary of the results arranged to show the shading effects is presented in Table II, which comprises the sums of eight values obtained for each of the three shading occasions, together with the differences to be ascribed to this treatment, the overall totals for treated and untreated plants, and the standard errors of the differences based on 24 degrees of freedom. Differences between the dry-weight values and the sum of the other two measured fractions, residual dry weight and total sugar, which represent the soluble material other than sugar, are also included.

A marked increase in mean values of dry weight and residual dry weight must accompany growth, but during the period here considered there is no evidence of larger differences between duplicates being associated with high mean values in the case of stems and sheaths, and analysis of variance of the original data would therefore seem an appropriate procedure for estimating errors. For ears, however, and for all sugar data, duplicate differences are related to the size of their means and it is recognized that in general such a relationship demands transformation of the data to equalize variance. In this particular case interest is confined to the additive effects of stem shading, and the comparisons to be made are between sums of values in which error variations associated with variations in the means are symmetrically distributed; consequently the estimate of error obtained from the untransformed data has been used. At the final collection sugar values had fallen to extremely low levels and data for this collection have been omitted from the statistical analysis.

Inspection of the treatment totals and their differences in Table II shows that shading the stem consistently reduces the mean level of dry weight, of residual dry weight, and of total sugar in all organs except the leaves. The main effect of shading is significant at the 0.01 per cent. level for all three constituents in stems and ears, and for dry weight and total sugar in sheaths, while in leaves the mean effects are quite negligible except for total sugar, where the observed mean difference just fails to reach the 5 per cent. significance level. It is also apparent that shading differences in the soluble fraction other than sugar nowhere approach significant proportions. The values for

<sup>1</sup> These parts will be referred to in the sequel as sheaths and as stems.

TABLE II

*The Effect of Shading the Stem on the Dry Weight, Residual Dry Weight, and Total Sugar of component Parts of the Barley. Sums of eight Values comprising duplicate Analyses of 24 Tillers (four from each of six plants) made 7, 17, 35 Days after Shading and at normal Harvest Time.*

$s$  = control.  $S$  = stems shaded.  $s-S$  = differences due to shading. Sowing date, 19.5.42

| Time of shading<br>(days after<br>sowing). | Leaves.  |            |           | Flag-leaf sheath. |            |  | Stems.   |            |            | Ears.    |            |            |
|--|----------|------------|-----------|-------------------|------------|--|----------|------------|------------|----------|------------|------------|
|  | <i>s</i> | <i>s-S</i> |           | <i>s</i>          | <i>s-S</i> |  | <i>s</i> | <i>s-S</i> |            | <i>s</i> | <i>s-S</i> |            |
|  |          | <i>S</i>   |           |                   | <i>S</i>   |  |          | <i>S</i>   |            |          | <i>S</i>   |            |
|  |          |            |           |                   |            | <i>Dry weights (gm.)</i>                 |          |            |            |          |            |            |
| 50   | 38.47    | 36.51      | 1.96      | 17.62             | 13.82      | 3.80                                     | 125.83   | 101.04     | 24.79      | 85.54*   | 67.92*     | 17.62      |
| 66   | 31.51    | 34.27      | -2.76     | 21.40             | 19.88      | 1.52                                     | 143.56   | 123.35     | 20.21      | 136.69   | 125.34     | 11.35      |
| 80   | 30.05    | 29.17      | 0.88      | 19.73             | 19.45      | 0.28                                     | 146.31   | 134.88     | 11.43      | 190.34   | 183.42     | 6.92       |
| Totals                                     | 100.03   | 99.95      | 0.08±2.65 | 58.75             | 53.15      | 5.60±1.32                                | 415.70   | 359.27     | 56.43±12.7 | 412.57   | 316.68     | 95.89±11.7 |
|  |          |            |           |                   |            | <i>Residual dry weights (gm.)</i>        |          |            |            |          |            |            |
| 50   | 25.95    | 25.00      | 0.95      | 11.59             | 10.28      | 1.31                                     | 91.02    | 72.19      | 18.83      | 71.65*   | 56.44*     | 15.21      |
| 66   | 22.21    | 23.82      | -1.61     | 15.20             | 14.75      | 0.45                                     | 107.10   | 90.92      | 16.18      | 117.45   | 108.20     | 9.25       |
| 80   | 21.51    | 20.74      | 0.77      | 14.81             | 15.05      | -0.24                                    | 114.11   | 105.65     | 8.46       | 166.36   | 157.93     | 8.43       |
| Totals                                     | 69.67    | 69.56      | 0.11±1.96 | 41.60             | 40.08      | 1.52±0.94                                | 312.23   | 268.76     | 43.47±10.6 | 355.46   | 322.57     | 32.89±10.2 |
|  |          |            |           |                   |            | <i>Total sugar (mg.)</i>                 |          |            |            |          |            |            |
| 50   | 3788     | 2722       | 1066      | 4312              | 2170       | 2142                                     | 26588    | 17715      | 8873       | 7622*    | 5104*      | 2518       |
| 66   | 1371     | 1540       | -169      | 4103              | 2562       | 1541                                     | 27154    | 20378      | 6776       | 9230     | 7822       | 1408       |
| 80   | 605      | 801        | -196      | 2179              | 2097       | 82                                       | 19464    | 16358      | 3106       | 9358     | 9167       | 191        |
| Totals                                     | 5764     | 5063       | 701±369   | 10594             | 6829       | 3765±389                                 | 73206    | 54451      | 18755±4342 | 26210    | 22093      | 4117±1317  |
|  |          |            |           |                   |            | <i>Soluble non-sugar (by difference)</i> |          |            |            |          |            |            |
| 50   | 8.73     | 8.79       | -0.06     | 1.72              | 1.37       | 0.35                                     | 8.22     | 11.14      | -2.92      | 6.27*    | 6.38*      | -0.11      |
| 66   | 7.93     | 8.91       | -0.98     | 2.10              | 2.57       | -0.47                                    | 9.31     | 12.06      | -2.75      | 10.01    | 9.32       | 0.69       |
| 80   | 7.93     | 7.63       | 0.30      | 2.74              | 2.30       | 0.44                                     | 12.74    | 12.88      | -0.14      | 14.63    | 16.33      | -1.70      |
| Totals                                     | 24.59    | 25.33      | -0.74     | 6.56              | 6.24       | 0.32                                     | 30.27    | 36.08      | -5.81      | 30.91    | 32.03      | -1.12      |

Estimates of error based on 24 degrees of freedom for dry weights and residual dry weights of leaves, sheaths, and stems, and on 18 for ears and all sugar data. Heavy type denotes differences significant at 0.01% level or longer odds. Italics denote significant interaction between stem shading and the date of shading.

\* Sums of 6 only. Ears too small for separate analysis, 7 days after shading at day 50.

this fraction tend to be higher in the plants with shaded stems than in control plants, but this is only consistently so in the stems themselves. Important effects of stem shading are thus everywhere confined to residual dry weight and total sugar and will be discussed in terms of these two fractions. Additional data might serve to demonstrate a small effect on other soluble material, particularly in the stems. The differential effect of stem shading on the several organs is associated with their stage of development at the time of application of the shades. Leaves reached their maximum dry weight about 60 days after sowing, a few days after the first occasion of shading, so that dry weight and sugar content were falling throughout almost all the experimental period and the mean effects were restricted to a small difference in sugar level. Sheaths reached their maximum dry weight 75 days from sowing, that is between the second and third shading occasion, and here mean sugar level was markedly lowered, but mean residual dry weight not significantly so. Stems reached a maximum about the 85th day and in these organs both mean sugar and mean residual dry weight were considerably reduced, the same being true for the ears. Stem shading may therefore affect all parts of the plant, but the effects are largest when treatment is given while active growth is still proceeding.

The nature of the interaction of *S* and *O* is indicated by the differences between treated and control plants for each of the three treatment occasions in Table II. There is a consistent diminution in the shading effect as the interval between sowing and treatment increases from 50 to 80 days, for both residual dry weight and total sugar in all organs except the leaves which show a consistent trend only in the sugar differences. Here there is a significant interaction with time of shading, arising from the fact that the sugar level in the leaves is lowered after early shading and is slightly raised after late shading. In the sheaths this interaction is significant for total sugar at the 1 per cent. level, but here the shading effect is halved between the 50th and 66th days and quite negligible by the 80th day. On the other hand, the residual dry weight of the sheath is unaffected except after treatment on the 50th day. In stems and ears, in spite of the consistent nature of the results, the 5 per cent. level of significance is not reached for the interaction between shading (*S*) and occasion (*O*), except in the case of the sugar of the stem. It may therefore be concluded that shading the stem produces some reduction in both residual dry weight and total sugar on all three occasions, but that the effect is small when shading is delayed until the 80th day. This latter point possibly requires further confirmation in the case of residual dry weight. The effect of the time of application of the shading on the response of each organ is thus in general similar to the mean responses of the different organs, that is, the magnitude of the effects depends on the stage of development at which shading is begun, the effects diminishing as each organ approaches maturity (maximum dry weight).

Data for the separate collections are shown graphically in Figs. 2-5, where duplicate totals are plotted against collection dates. In the leaves residual

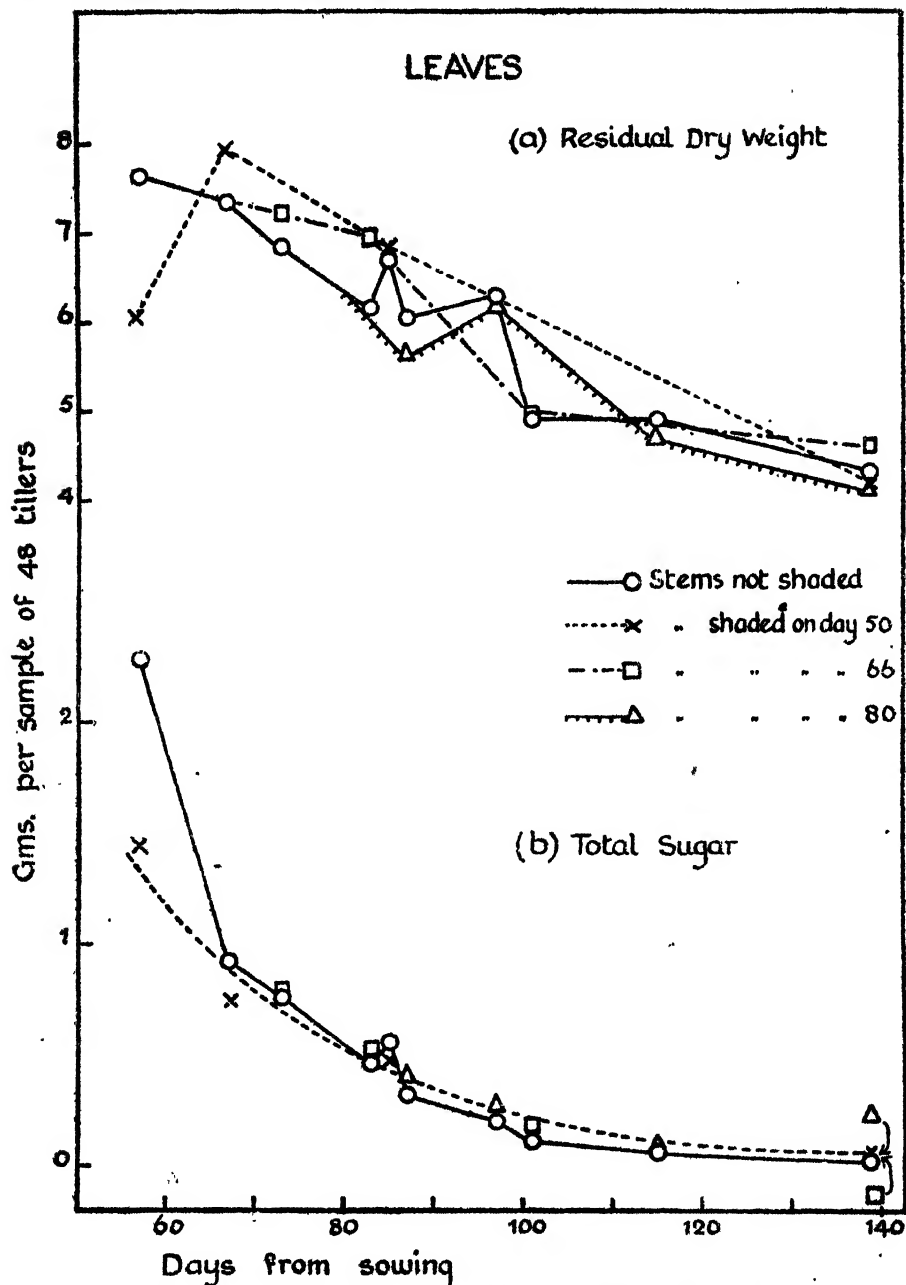


FIG. 2. Residual dry weights (a) and total sugars (b) in leaves of barley after shading the stems on three occasions. The values are sums of duplicate results. Samples consisted of four tillers from each of six plants. The dotted line shows exponential curve of closest fit through the figures obtained after shading on the 50th day. Corresponding curves for the other two shading occasions are omitted as they fall too close to the control figures to be readily distinguishable (see Table III).

dry weights (Fig. 2a) show no consistent differences between treated and control plants, so that coupled with the absence of both a mean effect and any interaction between *S* and *O* it is clear that leaf residual-dry-weight is unaffected by stem shading throughout the period here considered; in other words, the normal breakdown of this fraction, which begins a few days after the first treatment occasion, proceeds unchanged. Fig. 2b shows that the low mean sugar level after early shading is brought about by one low value in the stem-shaded plants 7 days after treatment. For the two late-shading occasions and for the later collections of the first occasion sugar values are higher in treated than control plants, and relative rates of sugar loss from the leaves, as calculated from exponential curves of closest fit, are significantly lower than the control value for all three treated series. These data are presented in Table III together with the sugar maxima for the several organs.

TABLE III

*The Effect of Shading the Stems on maximum Sugar Content and the Relative Rate of Sugar Loss in Barley. Sowing date, 19.5.42*

|  | Leaves.      | Flag-leaf<br>sheaths.    | Stems and sheaths<br>other than that<br>of flag leaf. | Ears. |
|--|--------------|--------------------------|---|-------|
| Time of maximum (days after<br>sowing)                               | 56           | 73                       | 85  | 85    |
| <i>Approximate maximum sugar content<br/>(gm. in 48 tillers)</i>     |              |                          |   |       |
| Not shaded   | 2.2          | 2.0                      | 12.0  | 6.0   |
| Stem shaded on 50th day  | 1.4          | 1.0                      | 9.5   | 4.0   |
| "    "    66th "   | past max. by | 1.6                      | 8.5   | 3.6   |
| "    "    80th "   | 66th day     | past max. by<br>80th day | 11.0  | 5.0   |
| <i>Relative rates of sugar loss*<br/>(mg. per gm. sugar per day)</i> |              |                          |   |       |
| Not shaded   | 58           | 89                       | 91  | 70    |
| Stem shaded on 50th day  | 41†          | 51†                      | 88  | 57†   |
| "    "    66th "   | 48†          | 76                       | 77  | 59    |
| "    "    80th "   | 45†          | 91                       | 85  | 72    |

\* Calculated from exponential curves of closest fit.

† Significantly below control value.

The low initial value for the first treatment occasion is associated with the short period following treatment during which sugar continues to rise in control plants. This value is relatively (per cent. dry weight) as well as absolutely low, while corresponding figures for residual dry weight show an absolute (Fig. 2a) but no relative difference. There is thus some evidence of a real difference in sugar content at this stage, indicating failure to maintain the normal sugar level in leaves of plants treated before the sugar maximum in this organ is reached.

In the sheaths residual dry weights (Fig. 3a) are low throughout after early shading; the difference between control and treated plants arises during the



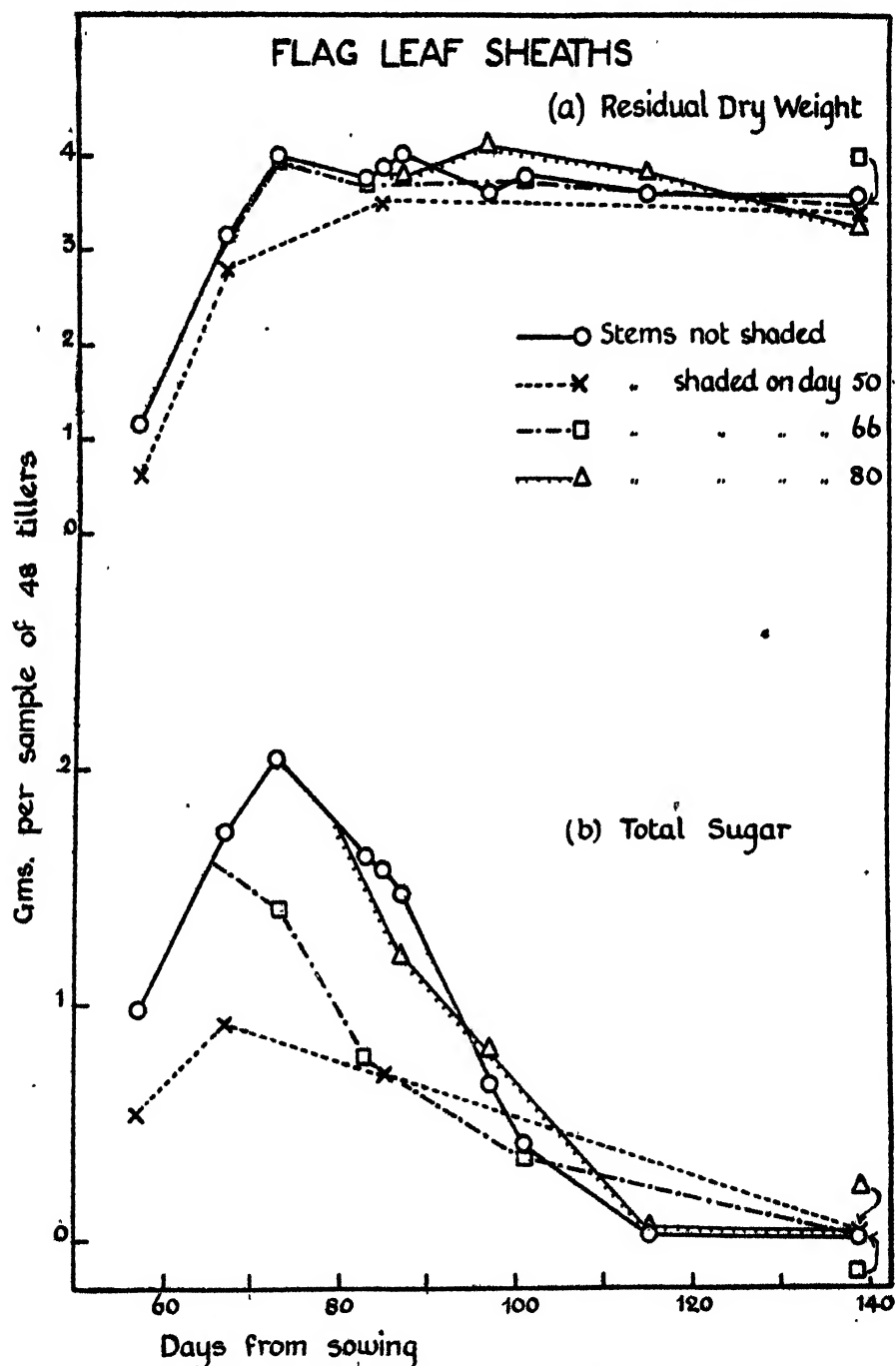


FIG. 3. Residual dry weights (a) and total sugars (b) in flag-leaf sheaths of barley after shading the stems on three occasions. Values as in Fig. 2.

time in which this fraction is still normally increasing. There is no effect at any stage after the two late-shading dates. The association of shading effects with periods of rapid growth is thus again emphasized, the effect being a restriction of normal growth. Sugar values in the sheaths (Fig. 3*b*) continue to rise slowly for a short time after stem shading on the 50th day, but further increase is prevented when the shading is on the 66th day. By the 80th day sugar has already passed its maximum and the normal downward drift is unaffected by treatment. The relative rates of loss during the falling phase are included in Table III. Only the value for the first shading occasion is significantly below that of the control. The maxima following both first and second occasions, however, fall short of the control maximum so that the tendency towards reduction in relative rate of sugar loss is accompanied by a reduction in the absolute rate of loss as a result of stem shading at these times.

In the stems themselves the treatment effect on residual dry weight (Fig. 4*a*) is similar to that on the sheaths except that, associated with the later attainment of the normal maximum, the level is reduced after treatment on both the 66th and 50th days. Again, differences after the latest treatment date are insignificant. In the ears (Fig. 5*a*) the diminishing effect of stem shading with the advancing treatment date is once more evident. Marked differences in sugar level in the stems are found within a week of shading on the first two occasions; but subsequently sugar accumulation is resumed at normal rates, as is shown by the similar slopes of lines representing the rise of sugar content in the two treated sets and in the control (Fig. 4*b*). The maxima reached by the treated stems fall below the control value owing to the initial difference set up immediately following shading, and these low maxima lead to reduced absolute rates of loss during the phase of falling sugar content, although relative rates of sugar loss from the stems are unaffected by shading (Table III). There is no significant effect of shading on sugar content following shading on the 80th day, and the effects on the sugar of the ear are for all three occasions in all respects similar to those on the stems themselves. The present data are inadequate to show whether the lower sugar levels in the stems and ears after stem shading are merely the result of a temporary restriction in sugar accumulation, or whether there is some actual loss of pre-existing sugar before the resumption of normal accumulation.

The general effect of stem shading may be summarized in the statement that there is some reduction of both polysaccharide synthesis and sugar storage not only in the stems themselves but in other parts of the plant still growing when the treatment is applied. On the other hand, polysaccharide breakdown is not induced nor is sugar loss accelerated when stems are shaded at the time of maximum dry weight; indeed the temporary failure to accumulate sugar after early shading tends to reduce the subsequent rate of loss in the senescent phase rather than increase it. In general, therefore, reduction of carbohydrate supply by this means inhibits the normal synthetic processes associated with growth but does not induce changes in normal degradative processes in mature organs.

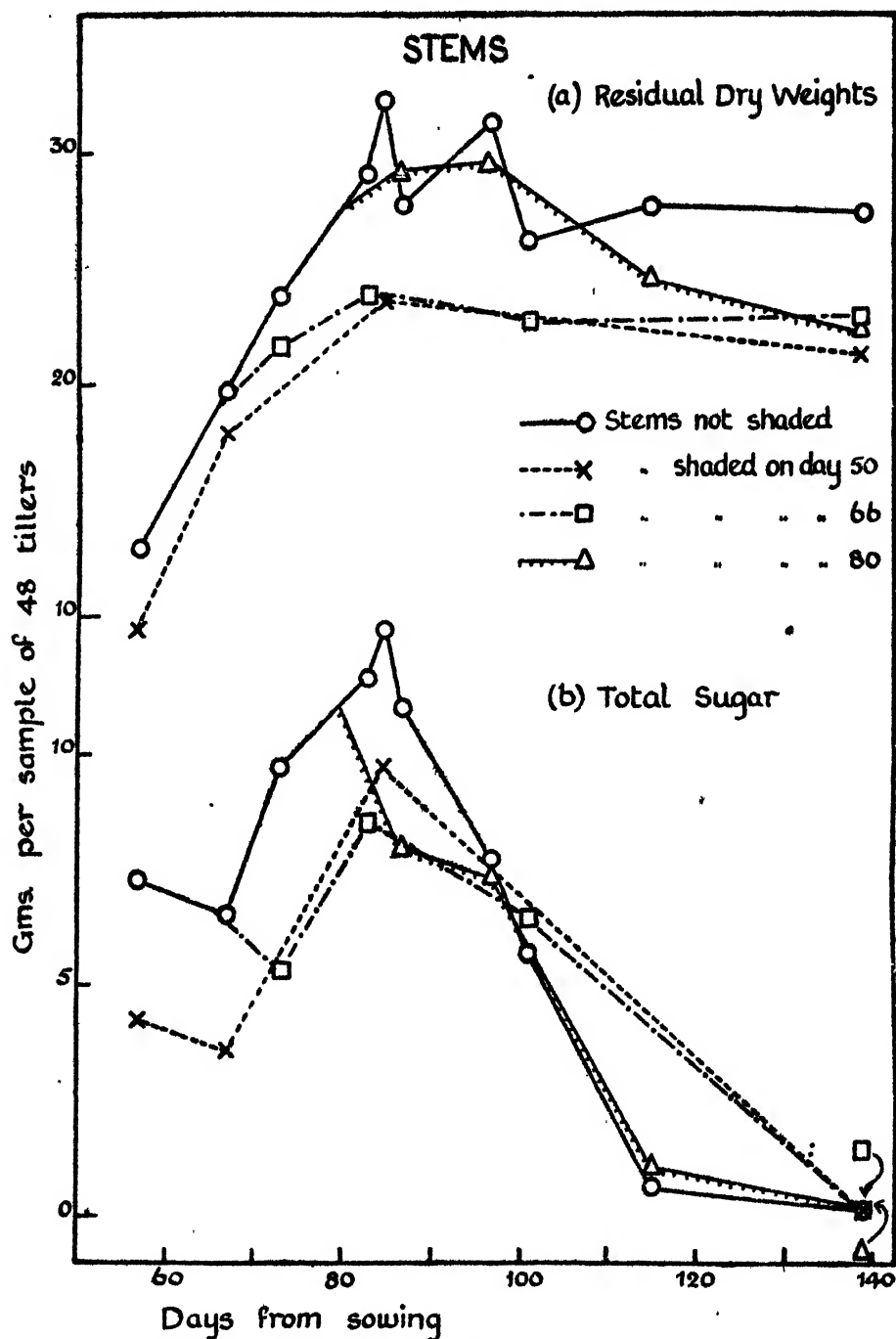


FIG. 4. Residual dry weights (a) and total sugars (b) in stems of barley after shading the stems on three occasions. Values as in Fig. 2.

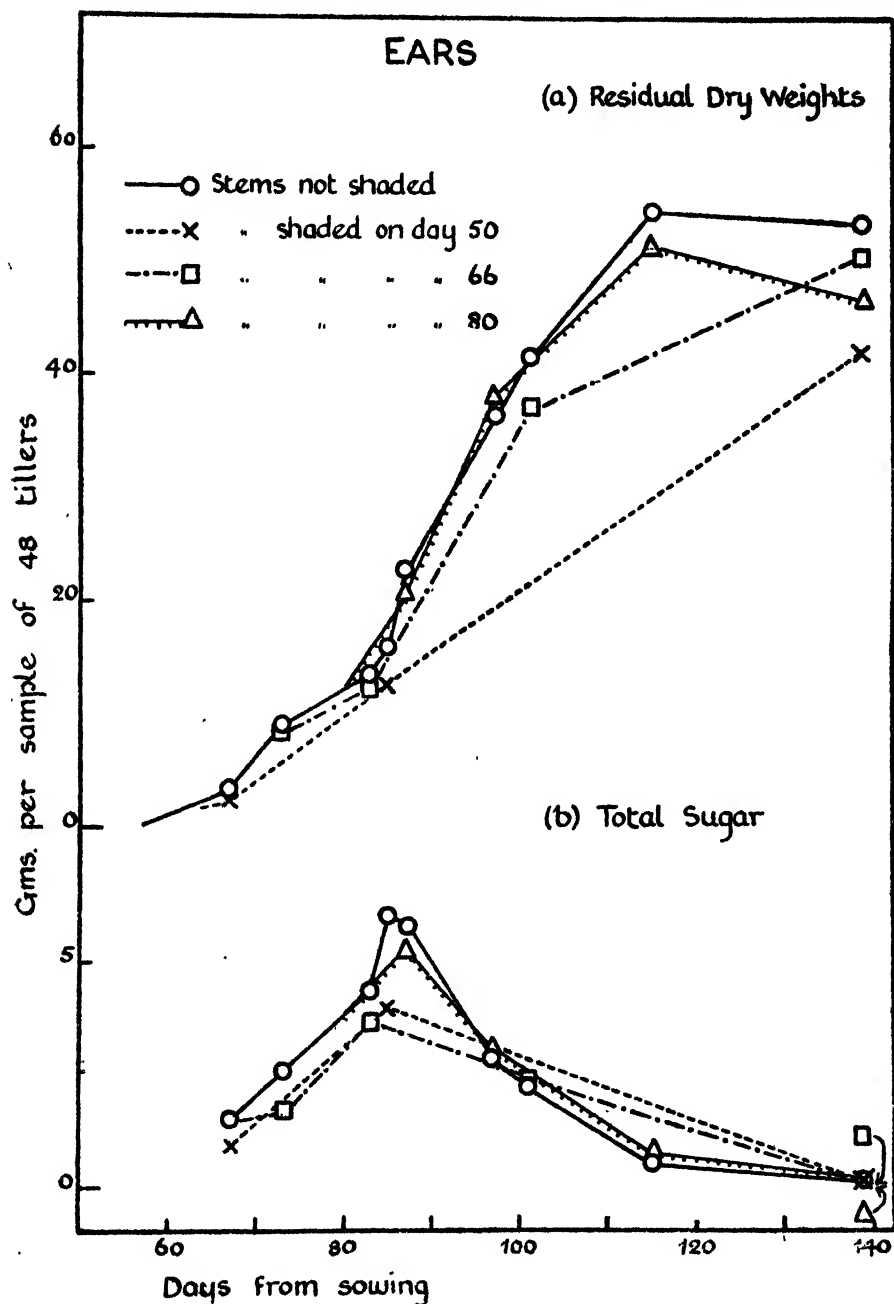


FIG. 5. Residual dry weights (a) and total sugars in ears of barley after shading the stems on three occasions. Values as in Fig. 2.

## EFFECT OF EAR REMOVAL AND DEFOLIATION

Groups of 8 pots were treated 66 days after sowing. Ears were removed from the four largest tillers of each plant of one group, leaves from a second group, both ears and leaves from a third, and the fourth served as a control. The ears were removed by making a slit in the sheath and severing the ear from the peduncle, but leaving it in place to avoid damage in its withdrawal. At the time of treatment awns were beginning to show on the main axes; sugar in the stems had reached half the maximum value, and the total stem dry weight three-fifths of the maximum. During the ensuing 3 weeks ears emerged on control and defoliated plants and stem sugar attained its maximum. The treatment period thus covers the final stage of stem growth and nearly the whole (11/12ths) of the growth cycle of the ear. As a result of ear removal the later formed tillers which would normally have failed to produce ears now did so, while in some pots new tillers appeared. In the earless tillers further growth of the stem was inhibited, and after an interval the sheaths began to turn yellow progressively from the tip downwards. There appears therefore to have been migration of nitrogen following this treatment.

The analytical data, as in the previous experiment, consist of duplicate determinations of dry weight, residual dry weight, and total sugar in samples from each of the four groups of plants, collected on four dates, making eight observations for each constituent. Sums of these sets of eight values and the main effects of ear removal and defoliation together with their standard errors are presented in Table IV, which also includes estimates of soluble material other than sugar obtained by difference. Sampling errors (chiefly due to differences in fresh weight) are unusually high in this material and as a result only the sugar contents show clearly defined treatment effects; residual-dry-weight differences are significant only for defoliated stems, and as in the previous experiment those for soluble non-sugar nowhere approach significance. Defoliation reduced the sugar levels throughout the plant, and certainly reduced the residual-dry-weight level in the stems, while ear removal led to a general increase in sugar level. Data for the separate collections for residual dry weight and total sugar in stems and sheaths are shown graphically in Figs. 6 and 7, where duplicate sums are plotted against time from sowing. Examination of the residual-dry-weight curves (Fig. 6) suggests that defoliation restricts further increase in this fraction, while ear removal is without effect on the sheath residual dry weight but again restricts growth in the stems, an observation borne out by the appearance of the plants in the field. In view of the large errors, however, these suggestions require some confirmation.

The treatment effects on total sugar, which are not in doubt, are on the whole similar in sheaths and stems. Earless tillers continue to accumulate sugar, reaching higher maximum levels than normal tillers, but at about the same time. Defoliated tillers either lose sugar continuously (sheaths) or accumulate further sugar very slowly (stems), reaching a low maximum value.

TABLE IV

*The Effect on Barky of Defoliation and of Ear Removal, 66 days after Sowing. The data represent the sums of duplicate determinations on four collection dates and are expressed as the amount (gm.) in 192 (24×8) tillers.*

*C = Control, F = Defoliated, Er = Ears removed, ErF = Ears and Leaves removed.*

|                     | Leaves. |       | Flag-leaf sheaths. |       |       |       | Stems. |        |        |        | Ears. |       |
|---------------------|---------|-------|--------------------|-------|-------|-------|--------|--------|--------|--------|-------|-------|
|                     | C       | Er    | C                  | F     | Er    | ErF   | C      | F      | Er     | ErF    | C     | F     |
| Dry weight . . .    | 36.61   | 39.99 | 22.19              | 17.92 | 22.14 | 19.81 | 133.68 | 109.45 | 144.28 | 113.91 | 92.37 | 71.61 |
| Residual dry weight | 25.98   | 27.50 | 14.27              | 12.67 | 14.16 | 13.91 | 99.63  | 81.48  | 93.40  | 84.79  | —     | —     |
| Total sugar . . .   | 2.67    | 3.75  | 5.64               | 3.01  | 6.16  | 3.94  | 26.91  | 13.68  | 37.66  | 19.92  | —     | —     |
| Soluble non-sugar . | 7.96    | 8.74  | 2.28               | 2.24  | 1.82  | 1.96  | 7.14   | 14.29  | 13.22  | 9.20   | —     | —     |

*Main Treatment Effects (×16) with their Standard Errors based on 16 Degrees of Freedom.*

|                     | Leaves.    |  | Flag-leaf sheaths. |   |            |     | Stems.       |   |              |     | Ears.       |   |
|---------------------|------------|--|--------------------|---|------------|-----|--------------|---|--------------|-----|-------------|---|
|                     | Er         |  | C                  | F | Er         | ErF | C            | F | Er           | ErF | C           | F |
| Dry Weight          | +3.38±2.21 |  | —6.60±1.98         |   | +1.84±1.98 |     | —54.60±14.61 |   | +15.06±14.61 |     | —20.76±7.49 |   |
| Residual dry weight | +1.52±1.46 |  | —1.85±1.48         |   | +1.13±1.48 |     | —26.76±10.40 |   | —2.92±10.40  |     | —           |   |
| Total sugar . . .   | +1.08±0.36 |  | —4.72±0.54         |   | +1.32±0.54 |     | —30.97±4.05  |   | +16.98±4.05  |     | —           |   |
| Soluble non-sugar . | +0.78      |  | —0.03              |   | —0.61      |     | +3.13        |   | +1.00        |     | —           |   |

*Italics denote effects significant at the 5 per cent. level or longer odds.*

Tillers without ears or leaves show intermediate values between controls and those only defoliated. After the maxima are reached sugar levels decline, eventually approaching zero values, irrespective of treatment, at about the time ears normally reach their maximum dry weight (115 days from sowing).

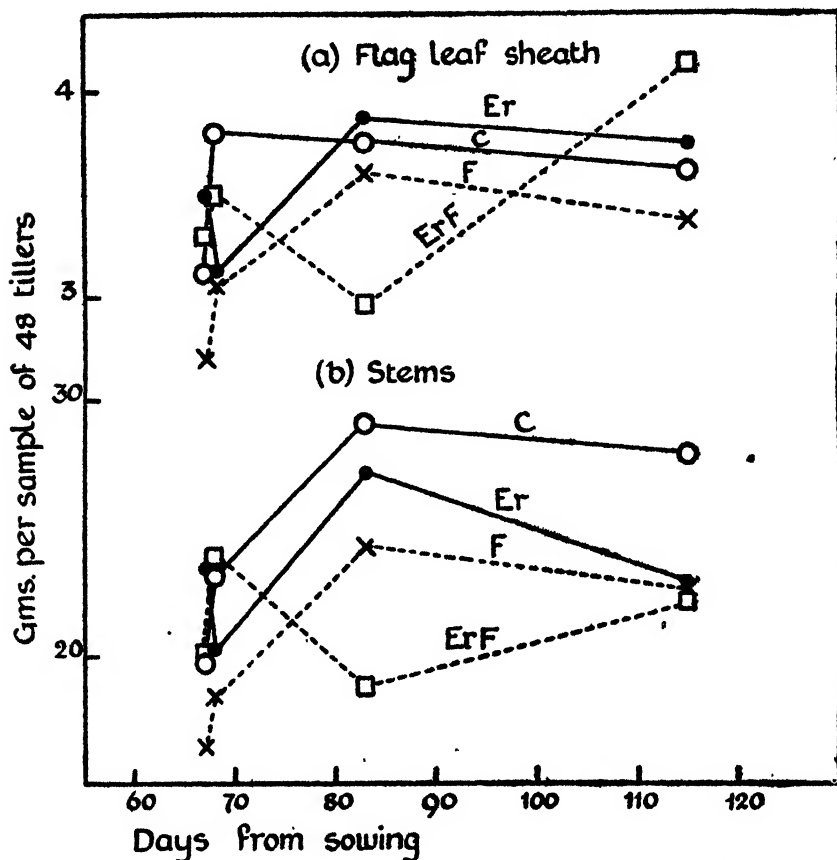


FIG. 6. Residual dry weights of (a) flag-leaf sheaths and (b) stems of barley after defoliation (F), ear removal (Er), and both treatments together (ErF), together with controls (C). Treatments given 66 days after sowing as awns beginning to show. Values as in Fig. 2.

The rate of sugar loss with these treatments as well as with stem shading therefore depends primarily on the maximum attained. In support of the view that sugar loss is independent of ear requirements, it may be emphasized that earless tillers show the same time sequence of sugar change as normal tillers, and in consequence the maximum sugar level reached is high and the ensuing rate of loss actually greater than in normal stems.

The low initial values for sugar content in defoliated stems might suggest a considerable loss of sugar in the first 48 hours after treatment. Similar low values are found, however, for residual dry weight, and the high sampling

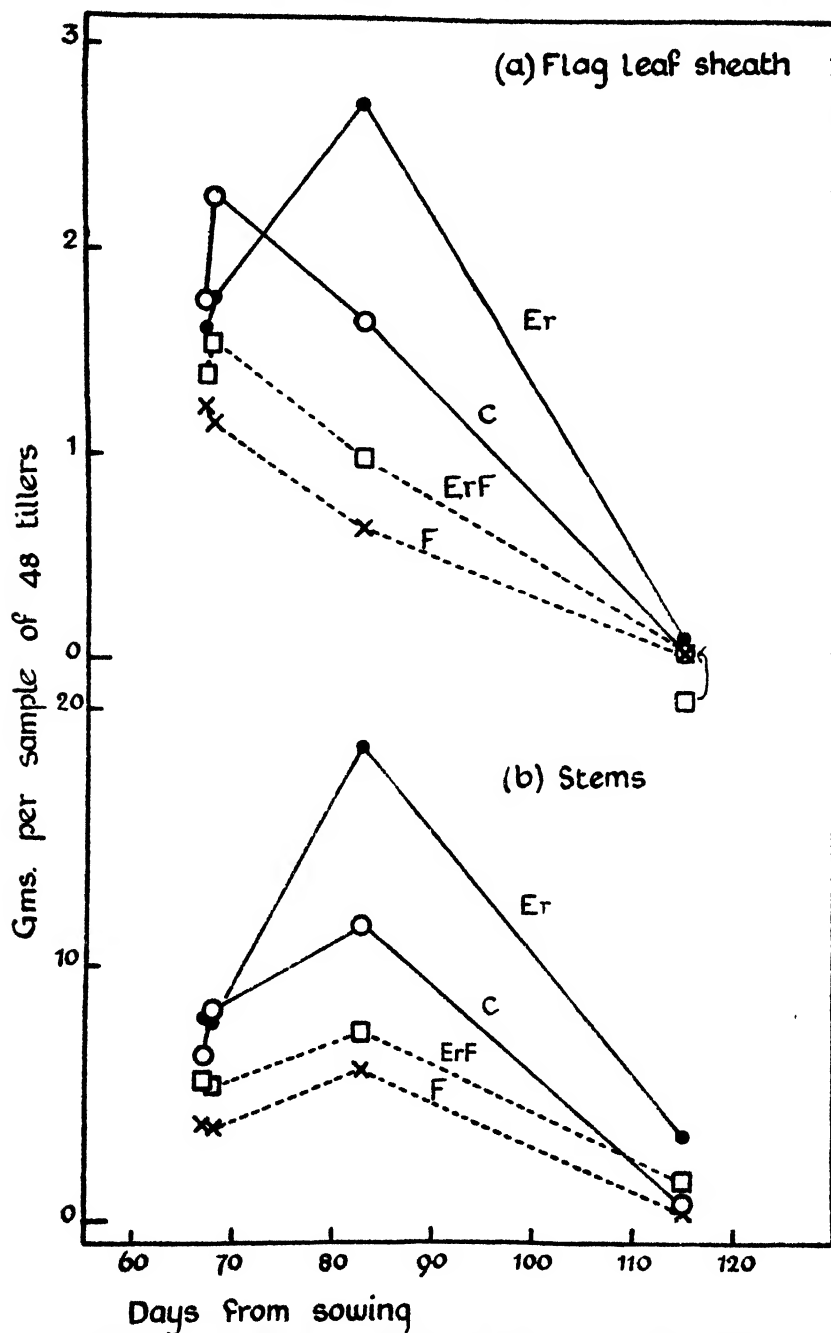


FIG. 7. Total sugar in (a) flag-leaf sheaths and (b) stems of barley after defoliation and ear removal. Treatments as in Fig. 6, and values as in Fig. 2.



errors coupled with the fact that earlier defoliation experiments gave no indication of such residual-dry-weight losses make further confirmation of the sugar data necessary before this interpretation can be accepted.

#### THE CONTRIBUTION OF THE COMPONENT PARTS TO THE DRY WEIGHT OF THE EAR

The percentage reduction due to stem shading in the dry weights of stems and ears at the final collection (139 days after sowing) are shown in Table V. The data for this final collection do not permit of very accurate estimates of

TABLE V

*Percentage Reduction at Harvest (139 days from sowing) in the Dry Weights of Stems and Ears of Barley as a Result of Shading the Stems*

| Shading date<br>(days from sowing). | Stems. | Ears. |
|-------------------------------------|--------|-------|
| 50                                  | 26     | 19    |
| 66                                  | 9      | 5     |
| 80                                  | 12     | 5     |

the shading effect, as may be seen in Fig. 5*a*, but taking into account the general trend of these curves of ear residual-dry-weight it seems unlikely that any effect on the ear could exceed 10 per cent. of the harvest weight with stem shading on the 66th day or later, while the reduction is of the order of 20 per cent. when shading is on the 50th day. In the defoliation experiment the dry weight of the ears averaged 1.10 gm. for control plants as compared with 0.80 for defoliated plants, a difference of 25 per cent. From the present experiments therefore the estimates of the contributions of leaves and stems to the dry weight of the ear are 25 per cent. for the leaves and only 10 per cent. for the stems after the 66th day, and another 10 per cent. for the stems between the 50th and 66th days. These values may be compared with estimates deduced from previous experiments with the 1940 crop (Archbold, 1942); in these the contribution of the stem was determined for four treatment-occasions, 60, 66, 74, and 81 days after sowing, using the difference between the ear growth made by control plants after the treatment date and that made by stems exposed to light but deprived of leaves and with the ears shaded. These four estimates lay between 20 and 30 per cent. per ear. On the other hand, defoliation alone on each of these four dates failed to reduce the dry weight per ear. There is thus considerable discrepancy between the results for the two seasons.

Additional but unpublished data now available for plants grown at two nitrogen levels showed for the lower (somewhat lower than that in the stem-shading experiment) level defoliation effects on the ears of 12 and 8 per cent. for treatment on the 60th and the 80th days. At the higher level the ear dry-weight was reduced by only 9 per cent. after early defoliation and there was no effect after the late treatment date. In spite of the differences between one experiment and another, such as season, sowing date, nitrogen manuring,

&c., the results are consistent in suggesting that if defoliation is delayed until ears are emerging on the four largest tillers (about the 80th day) reduction in the final dry weight of the ear does not exceed 10 per cent. and may be negligible. During the period of the filling of the grain, when 60 per cent. of the ear dry-weight accumulates, leaves appear therefore to play only a minor part as far as carbohydrate is concerned. The results of early defoliation are on the contrary complex, and both nitrogen supply and the precise time of treatment in relation to the age of the tiller play a part in determining the effect. Thus in 1940 defoliation on the 67th day reduced the number of ear-bearing tillers from 5.2 to 3.5 per plant but did not affect the mean dry weight per ear. While in 1942, under similar conditions with respect to nitrogen supply but with a later sowing date, the tiller number was reduced from 5 to 4.6 per plant, but as well, the average dry weight per ear was 25 per cent. less in the defoliated plants. In 1942, under conditions of more extreme nitrogen deficiency, leaf removal reduced tiller number from 4 to 3.6 per plant but only reduced by 12 per cent. the mean dry weight per ear. The implications of these data will be further discussed in a later publication; it suffices to note here that the nitrogen supply must be taken into account in studying the effects of early defoliation, and that in addition to the contribution made by the leaves after ear emergence amounts of the order of a further 15 per cent. are so contributed before emergence.

The discrepancies in the estimates of the stem contribution leave uncertain not only the magnitude of this contribution but also the time at which it is made. The results of stem shading suggest that after ear emergence, the stems are relatively ineffective in this respect, and make their maximum contribution between the 50th and 66th days; this view is supported by the observed effects of defoliation and ear removal on stem sugar. On the contrary, the data of the earlier experiments relating to stems left exposed to light, suggest that stems continue to supply material after ear emergence to the extent of 20 to 30 per cent. of the total ear dry-weight. Values of 10 per cent. for leaves and 30 per cent. for ears, both after emergence, obtained independently would, together with this larger estimate of the stem contribution, make up the 60 per cent. of the total for the ear which is accumulated after emergence. It follows that either the estimate derived from stem shading is too low or those for leaves and ears are at fault. It seems unlikely that the leaf contribution after ear emergence is grossly underestimated since the area of green leaf is rapidly diminishing at this time; the remaining possibility is that the ear itself under normal circumstances contributes more than the apparent 30 per cent. deduced from experiments with shaded ears. Additional evidence must be sought before a decision can be reached on this point. At present it is certain that the operation of secondary effects, other than prevention of assimilation by one organ or another, when parts of the plant are shaded or removed have obscured the measures of assimilatory capacity. The values quoted above must therefore be regarded as no more than rough approximations.

## DISCUSSION

The sugar metabolism of the barley stem may conveniently be divided into two phases; one is that of rising sugar content during which the stem elongates and the ear develops within the sheath, the other that of falling sugar content in which the ears emerge and stem growth is complete. It is generally supposed that the late 'shooting' of the ear results in excess carbohydrate accumulating in the stems during the first phase, and that this sugar is translocated upwards to the ear during the second phase.

By eliminating in turn, either by shading or removal, the three principal assimilating organs, namely leaves, ears (Archbold, 1942), and now stems, it was found that when these treatments were applied during the phase of rising sugar only defoliation had a major effect in restricting sugar accumulation. Leaf metabolism, as judged by the absence of effect on leaf composition, was unaffected by shading of stems or ears, so that on the assumption that with these two treatments leaves continue to supply sugar to the stem in a normal manner, it follows that the assimilation of the leaves is mainly responsible for the sugar accumulating in the stem. This view is borne out by the behaviour not only of shaded stems but of stems of defoliated plants. After defoliation stems were able only to maintain or to increase slightly the low sugar levels found two or three weeks prior to the sugar maximum; and if ears were removed also the position remained unaltered except for a slightly raised sugar level. Finally ear removal in the presence of leaves led to accumulation of large amounts of sugar in the stems, so that failure of stems alone to accumulate sugar suggests that their assimilatory capacity is not high (see also p. 381).

The several treatments designed to restrict the carbohydrate supply had effects upon polysaccharide synthesis as well as upon sugar content; in general all three types of treatment tried tended to restrict in some degree this synthesis. The problem is not, however, simply one of supply and demand as determined by the available carbohydrate, since with stem shading or ear removal there is partial or complete inhibition of residual-dry-weight increase in both stem and sheath in the presence of excess sugar and unchanged nitrogen supply. Only in defoliated plants is the sugar at really low levels, and here removal of nitrogen with the leaves may also be a factor restricting growth. In the case of earless tillers there is some evidence, from the appearance of new tillers and the gradual yellowing of the sheath, that nitrogen is exported as soon as the mobilizing influence of the ear is removed; but this reversal of the direction of nitrogen movement is not accompanied by that of sugar, which continues to accumulate to an increasing extent until the time of the normal onset of sugar loss. In the shaded stems the failure of normal lignification although extension growth is increased (Table I) is presumably associated with the absence of light. A continuous supply of carbohydrate from the leaves is thus not in itself sufficient to maintain normal stem growth; the ears must be present, possibly to regulate nitrogen supply, and the stems must be in the light.

The results of these experiments are clearly consistent with the view that, under normal conditions, the sugar accumulating in the barley stem is mainly the result of assimilation by the leaves in excess of their immediate requirements. The results also show that the actual utilization of the supply is itself controlled by a number of other factors associated with the regulation of growth, which may also be affected by treatments designed to reduce carbohydrate supply. Consequently these treatments do not necessarily lead to utilization of all available sugar before other effects on growth occur. It would seem that nitrogen and carbohydrate are continuously exported from the leaves during ear development, and that the fate of these exports depends on the stage of growth of the other plant organs, and may be modified by such treatments as those employed, but not in such a way as to indicate that 'demand' for carbohydrate by the ear exerts any controlling influence.

The rather sudden onset of sugar loss from the stems has frequently been associated with flowering, and it certainly occurs about the same time. The existence of a causal relationship between these two phenomena is, however, in doubt in view of the behaviour of earless tillers and the fact that sheaths begin to lose sugar before the flowering date and at the time of their maximum dry weight. The continued accumulation of sugar within an organ appears to bear some relation to its growth, but the relation must be complex since a wide range of ratios of sugar to residual dry weight is found with varying external conditions; moreover under abnormal conditions, such as the absence of ears, sugar accumulates in the stem when growth is prevented.

When defoliation and shading were delayed until the sugar level in the stems had reached its maximum and were therefore only operating during the second phase, that of falling stem sugar, the reduction of the current supply of assimilate failed to influence the rate of sugar loss. On the other hand, the lower maxima induced by early treatment were accompanied by reduced rates of sugar loss during the second and senescent phase. In addition to the absence of effect upon sugar loss normal loss of residual dry weight after the completion of stem growth was also unaffected by any of the three treatments. The conclusion already put forward (Archbold, 1942) that loss of sugar from the barley stem is unrelated to the filling of the grain receives support from the new data relating to shading of the stem presented here, and it is also clear that the rate of sugar loss is in large measure dependent on the maximum reached.

The principal result of the whole series of experiments involving defoliation and shading of the stems and ears is the evidence they provide that free sugars (fructosans, sucrose, fructose, and glucose) in the stems of barley are not precursors of polysaccharide in the ear or elsewhere, and that their loss from the stems is an inevitable senescent change and not a controlled movement in response to growth demands of the ear. While it seems clear that the stems are not storage organs in the sense that material previously stored is subsequently utilized elsewhere for growth, the actual fate of the sugar remains uncertain, and particularly how far the elimination is the result of respiration

(Archbold and Mukerjee, 1942). Coupled with the observations of the sugar changes is the fact that no major breakdown of polysaccharide occurs in any organ which would form a source of supply to the ear, nor is there major change on the soluble fraction other than sugar, which, if anything, tends to increase. The ear is therefore entirely dependent on primary assimilate translocated to it or produced *in situ* and immediately and irreversibly synthesized, if changes later associated with germination are left out of account. The material is supplied in turn by each of the plant organs, and finally the ear itself becomes the main agent in providing carbon for starch formation. It has already been pointed out (p. 381) that the operation of factors other than the prevention of assimilation tends to vitiate the estimates of the contribution of the several organs to the ear dry-weight, and that the evidence with regard to the stem is especially conflicting. Further speculation on the precise parts played by stem and ear would be premature.

The examples presented here of failure to utilize excess sugar for stem and ear growth emphasize the complexity of the problem of carbohydrate utilization and stress particularly the view that growth is controlled mainly by factors other than carbohydrate supply. The present experiments provide no evidence of a limiting sugar-supply except in defoliated plants. The general accumulation of sugar when ears are removed make it reasonably certain that these sugars or their immediate precursors are normally the substrate for ear growth. They may be excluded from further use, under conditions of artificially induced carbohydrate shortage, by their precise chemical form or by their location in a tissue which by reason of age has lost some requisite capacity for effecting translocation. Both age and nitrogen supply are obviously factors requiring study in relation to the problem of carbohydrate utilization. Experiments are now in progress to throw some light on the part played by nitrogen in determining the distribution of the available carbohydrate supply.

#### SUMMARY

The effects of shading the stems and of removing ears or leaves on the composition of the organs of barley has been studied. No significant effect of treatment on the fraction of the dry weight soluble in cold water, other than sugar, was observed. The data are therefore discussed in terms of the insoluble fraction 'residual dry weight' and total sugar. Stems were shaded on three occasions, 50, 66, and 80 days from sowing, and leaves or ears removed on one occasion 66 days from sowing.

Stem shading affected all the plant organs. This treatment while sugar was still increasing restricted both residual dry weight and sugar increases, but treatment at the time of the sugar maximum failed to induce polysaccharide breakdown or accelerate the rate of sugar loss. In stems and ears reduction of sugar level was brought about by a temporary check in sugar accumulation, from which there was complete recovery within a week, storage being resumed

at the normal rate. Relative rates of sugar loss were either reduced slightly or unaffected, so that absolute rates of loss depended primarily on the maximum reached and were low where these maxima were diminished by early treatment.

Defoliation prevented sugar accumulation and restricted residual-dry-weight increase, while ear removal led to a general increase in sugar level but also tended to inhibit residual-dry-weight increase in the stem. Tillers lacking leaves and ears behaved similarly to defoliated tillers, except that a slightly higher sugar level was maintained. Stem sugars in all treatments reached maxima at the same time and then fell to zero by about the 115th day from sowing. The high values reached in earless tillers thus led to rates of sugar loss even greater than in normal tillers, while in defoliated tillers, where the maxima were much depressed, rates of loss were low in spite of the depleted supply of carbohydrate.

The results of both experiments support the views put forward in earlier papers, firstly, that free sugars in the barley stem are not precursors of starch in the ear, and that their loss from the stem is an inevitable senescent change and not a controlled movement in response to growth demands of the ear; secondly, that major breakdown of polysaccharide does not occur, so that the ear is dependent upon primary assimilate immediately translocated to it or produced *in situ*. Since shaded or earless stems fail to make normal growth in the presence of increasing amounts of sugar, it appears that utilization of sugar depends mainly upon factors other than the carbohydrate supply.

The question of the amount of material supplied to the ear by assimilation in the stems is discussed. At present the available results are conflicting.

The authors have pleasure in acknowledging the continued help and unfailing encouragement received from Professor F. G. Gregory.

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# Experimental and Analytical Studies of Pteridophytes

## IV. Stelar Morphology: Experimental Observations on the Relation between Leaf Development and Stelar Morphology in Species of *Dryopteris* and *Onoclea*

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With Plate IV and Eighteen Figures in the Text

### INTRODUCTION

THE classical accounts of vascular structure in leptosporangiate ferns show clearly that the periodic local interruptions of the conducting cylinder are associated with the 'insertion' of the leaf-traces, i.e. the vascular strands of the petiole or leaf-base. Thus in a solenostelic fern a cross-section at the level where a leaf-base joins the shoot will show the presence of a leaf or foliar gap; in other words, the vascular cylinder which would otherwise be continuous is locally interrupted by non-vascular tissue, usually parenchyma. In shoots in which the leaves are inserted in close succession the leaf-gaps overlap and the vascular system in consequence becomes an open meshwork, or dictyostele, each individual strand being described as a meristele. Various tentative physiological and morphological explanations have been advanced to account for the development of these leaf-gaps (e.g. Jeffreys, 1903; Tansley, 1907). A considerable volume of literature also deals with the question as to what extent the vascular tissue of the shoot is of truly cauline origin or, alternatively, is a composite structure largely composed of decurrent leaf-traces. So far as the writer is aware, these problems have not been approached by direct experimental methods. An account will therefore be given of some simple and easily repeatable experiments, together with related observations, which throw new light on the relation between leaf development and stelar morphology.

In leptosporangiate ferns, as in pteridophytes generally, the initial differentiation of vascular tissue in shoot, leaf, and root takes place immediately below the meristem (Wardlaw, 1944). At the shoot apex the stele of a dictyostelic fern consists of an uninterrupted conical sheet of vascular tissue, in the initial stage of differentiation, enclosing a developing parenchymatous pith. A transverse section taken immediately below the apical meristem would thus show a complete ring of vascular tissue (Wardlaw, 1944a). Moreover, in ferns such as *Dryopteris aristata* or *D. filix-mas*, though five to seven separate vascular strands or leaf-traces are present in the fully developed leaf-base, the vascular system of a very young leaf primordium consists of a single newly



differentiated strand, elliptical in cross-section. This becomes conjoined with the shoot-stele, just below the shoot meristem, *no leaf-gaps being present at this early stage*. As the bilateral leaf primordium enlarges the vascular tissue becomes deeply crescentic or horseshoe-shaped as seen in transverse section, the free margins being thrust farther and farther apart by the enlarging parenchymatous pith. Concomitantly, in the region of conjunction with the shoot-stele, a small zone of developing parenchyma is now seen to interrupt the hitherto continuous ring of vascular tissue. On further enlargement the crescentic mass of vascular tissue in the leaf-base becomes sub-divided into five to seven or more strands separated by parenchyma, and the leaf-gaps in the shoot-stele become correspondingly widened and lengthened by parenchymatous development; and so on, until in the fully developed leaf-base the several vascular strands are widely thrust apart and the shoot-stele consists of a vascular meshwork, characterized by relatively small meristeles and large parenchymatous leaf-gaps. These facts, which are considered in greater detail elsewhere (Wardlaw, 1944a), suggest that leaf-gaps develop in those regions of the shoot-stele which are subject to mechanical stress as a result of the enlargement of the vascular systems of the leaf-bases; they also indicate that groups of cells, initially differentiated as vascular tissue and potentially capable of developing into phloem, xylem, &c., have been transformed during growth into parenchyma.

The hypothesis that a direct relationship exists between leaf development and the internal morphology of the shoot was thus based on observation of the normal changes during development. It therefore seemed cogent to inquire if experimental verification could be obtained. If the hypothesis was correct, then by suppressing or destroying a leaf primordium at a very early stage the formation of the corresponding leaf-gap should not take place; and if a succession of primordia were similarly destroyed, the vascular system of the shoot should develop not as a dictyostele but as a continuous uninterrupted cylinder. Moreover, if the treatment could be carried out when each leaf primordium had developed little beyond its initial stage, i.e. a single large meristematic cell, and if, nevertheless, a substantial shoot-stele developed, proof of the truly cauline nature of that stele would have been obtained for the species investigated.

Any other treatments which tend to modify the normal course of leaf inception and development may also throw light on the relationships under consideration. The experimental methods employed and the results obtained are given below.

#### EXPERIMENTAL TREATMENTS AND MATERIALS

The distal ends of stout erect shoots of *Dryopteris aristata* and *D. filix-mas* were completely defoliated and the scales removed so that the terminal region was left 'naked'. For these operations small forceps and scalpels were used. The apical meristematic cone was left intact, but the minute leaf primordia round its base were destroyed by needle-puncturing. For this a Zeiss micro-

manipulator was used, the material being observed under a binocular microscope. Alternatively, by using a needle on its side and gently moving it over the surface of the shoot away from the base of the apical cone it was possible to 'smooth out' all new leaf primordia without injuring the meristem. The apical region was then protected by means of moist cotton wool and the piece of shoot, trimmed to 2.0–4.0 cm. in length, placed in peat. Thereafter at weekly or fortnightly intervals any new leaf primordia which had appeared were destroyed. Roots were abundantly formed and some pieces of material were kept alive and growing for several months, until, in fact, the specimen was fixed for sectioning. Not only were new leaf primordia produced in considerable numbers but the shoot underwent a considerable elongation, Pl. IV, Fig. 3. The occasional lateral buds which developed were also obliterated. The materials were embedded in wax and sectioned from apex to base.

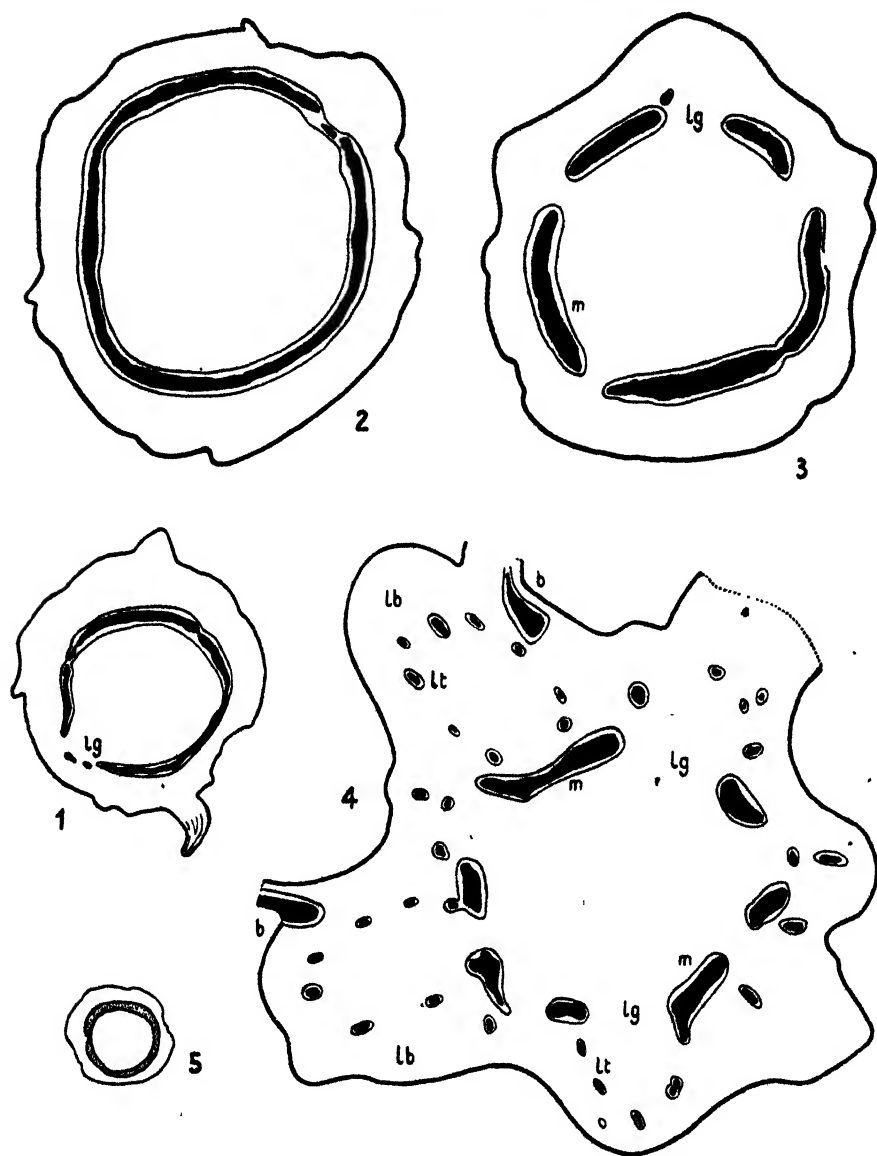
### RESULTS

In a majority of the specimens the apical meristems were found to be intact and apparently normal and every stage between complete solenostely and the normal dictyostelic condition of the older region of the shoot was observed (Text-figs. 1–5 and Pl. IV, Figs. 1, 4–6). Thus, where a succession of primordia had been destroyed immediately after their inception on the flanks of the apical meristem, a complete, uninterrupted vascular ring was observed. In due course this became fully differentiated, showed the normal distribution of xylem, phloem, &c., and, in fact, bore a close resemblance to the large solenosteles normally found in the rhizomes of certain ferns. The induced solenostele illustrated in Text-fig. 2 and Pl. IV, Fig. 1, is of large size, having a diameter of 0.8 mm. But where a leaf primordium had grown to a slightly larger size before it was destroyed a small leaf-gap developed, the width of the gap being more or less directly a measure of the 'diameter' (chord) of the leaf-trace crescent.

A comparison of Text-figs. 2–4 shows that the change from the dictyostelic condition in the older region to the solenostelic condition in the experimental region of the shoot was not attended by a reduction in stelar diameter. In fact, the diameter of the solenostele in this instance is slightly greater than that of the dictyostele. The solenostele is thus an acropetal extension of the dictyostelic cylinder, with the leaf-gap parenchyma replaced by vascular tissue; or, more precisely, tissue which was initially differentiated at the apex as vascular tissue has, in the absence of modifying factors associated with leaf development, become fully differentiated as vascular tissue.

In some specimens, during the last few weeks prior to fixing, the leaf primordia were not obliterated. These specimens showed solenostely in the treated region of the shoot and a return to normal dictyostely in the terminal region.

These experiments also throw light on the development of the foliar vascular system. During development the growth of the crescentic sheet of small-celled tissue does not keep pace with the enlargement of the pith within;



TEXT-FIGS. 1-5. *Dryopteris aristata*. Figs. 1-4. Series of transverse sections of a stout erect shoot from which all leaf primordia were systematically removed (see Pl. IV, Fig. 3). Fig. 4, lower region of shoot, fully developed and showing normal dictyostelic structure prior to experimental treatment; *m*, meristele; *lb*, leaf-base; *lt*, leaf-trace; *lg*, leaf-gap; *b*, bud. Fig. 3, taken higher up, in the region where leaf primordia were removed: because of the absence of the enlarged leaf-bases, shown in Fig. 4, the cross-sectional area is considerably smaller; the leaf-gaps of older leaves are beginning to 'close'. Fig. 2, still higher up: all leaf-gaps have disappeared and the vascular system is typically solenostelic; phloem and xylem are fully differentiated. Fig. 1, taken near the apex; the vascular ring is complete, except for a leaf-gap on the left-hand side. Fig. 5, complete, undifferentiated vascular ring below the apex of a smaller experimentally treated shoot. ( $\times 5$ .)

hence the vascular crescent becomes disrupted into five to seven or more separate strands or traces, these being still undifferentiated into phloem and xylem. As a result of the parenchymatous development which ensues, the small strands become widely separated in a horseshoe-shaped pattern. If, however, the further growth of the primordium is inhibited or prevented at an early stage, the normal enlargement of the pith does not take place and the crescentic mass of primordial vascular tissue remains coherent or disrupted to a limited extent only. Some of these points are illustrated in Pl. IV, Figs. 2 and 5. An examination of all the serial sections relating to Pl. IV, Fig. 5, shows that in this small primordium which is situated close to the shoot apex, the vascular tissue is typically trowel- or gutter-shaped and is not interrupted by panels of parenchyma.

#### EVIDENCE FROM ANOMALOUS LEAF-TRACES

Data from other experimental *Dryopteris* materials which throw light on the problem may now be considered. These relate to the influence exerted by certain anomalous leaf-traces on the development of the shoot-stele. It has been seen that the development of a leaf-gap is probably due, in the first instance, to the mechanical stress imposed on a local region of the developing vascular cylinder by the enlargement of the vascular system in the leaf-base. Now, it is conceivable that the vascular system of a leaf-base, under special circumstances or in relation to certain treatments, might *not* develop in the form of an ever-widening crescent; it might indeed develop to considerable size but in such a manner that its insertion on the shoot-stele did not subject the latter to stress and thereby lead to the typical parenchymatous development; i.e. a well-developed leaf-trace would become confluent with the shoot stele without a foliar gap. Materials which exemplify such a state of affairs have come under observation during the course of these investigations and may now be described.

A stout shoot of *Dryopteris filix-mas* was completely defoliated down to the smallest primordia. In the course of 5 weeks, during which the shoot was maintained in moist peat in a cool greenhouse, a number of new primordia had appeared and these also were destroyed. During the next 5 weeks a terminal leafy bud developed, consisting of 21 leaf primordia; immediately below there was evidence of bud development.<sup>1</sup>

The serial transverse sections which were prepared showed that a majority of the new primordia were normal in structure, though a few were of small size; leaf 19 (leaf numbers are given in basipetal succession) was small and necrosed, leaves 20 and 21 were normal; leaves 22, 23, &c., were those which had been removed. It may be inferred that leaves 19, 20, and 21 constituted a 'whorl' of very young primordia left after the second defoliation, leaf 19 having been damaged. Leaves 16, 17, and 18—the first to be formed after the experimental treatment—showed interesting anomalous developments.

<sup>1</sup> This treatment was applied in an attempt to induce bud development (see Wardlaw, 1943).

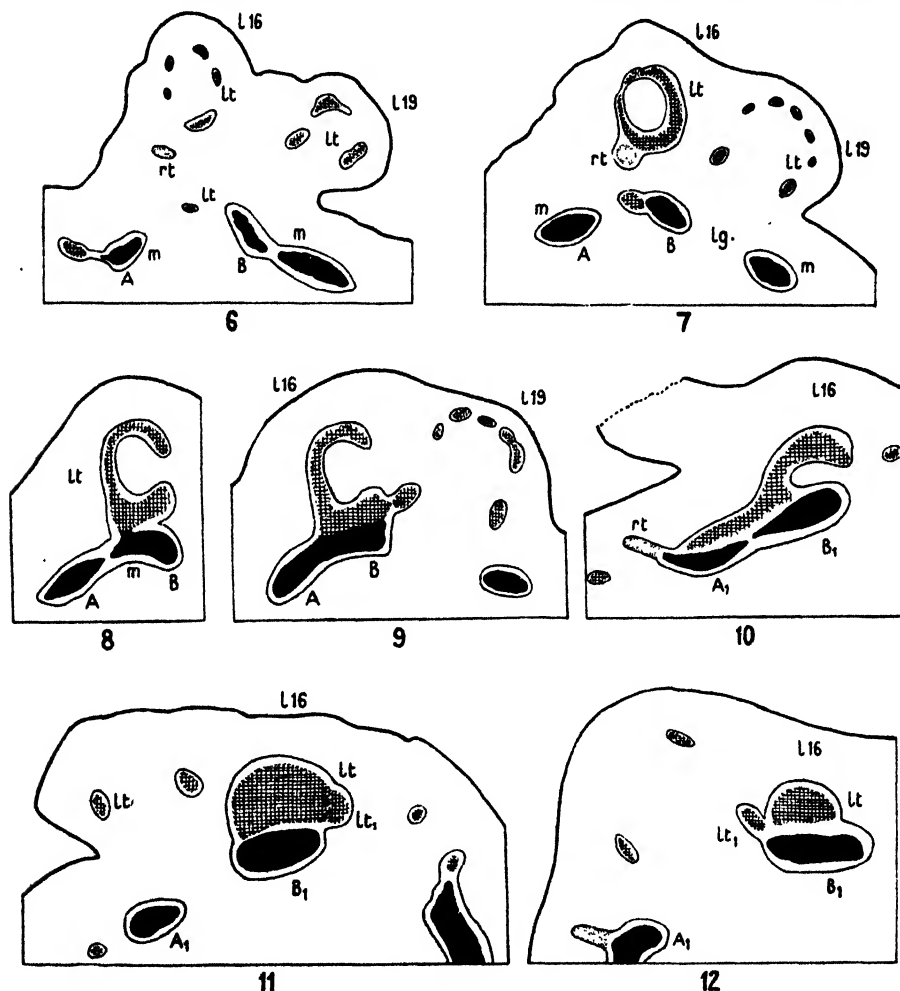
Leaves 17 and 18 were of small size; leaf 16 was of approximately normal size but had so developed that its adaxial side was at right angles to the surface of the shoot. The vascular system of this leaf—which can be traced to the intact apex—shows quite remarkable developments (Text-figs. 6–12).

At the level where the leaf-base joins the shoot the leaf-traces, instead of having the usual crescentic distribution, are disposed in the form of a ring (Text-fig. 6). Below this level the strands are no longer separate, the vascular system being essentially solenostelic (Text-fig. 7, Pl. IV, Fig. 7). Near the point of conjunction with the shoot-stele this foliar solenostele becomes partially disrupted on its abaxial face, first on one side and then on the other, but the essential cylindrical arrangement is retained. It becomes attached to the shoot-stele at the lower end of the leaf-gap of a younger leaf, i.e. it becomes confluent with two meristemes at their point of conjunction (Text-fig. 8). Now it will be seen that at this level the leaf-trace is C-shaped and is attached to the outer face of the meristeme along the base of the C, the xylem of the two systems becoming confluent (Text-fig. 9, Pl. IV, Fig. 8). By following the distribution of the vascular tissues downwards the remarkable fact is disclosed that no leaf-gap is present (Text-figs. 10–12). Where the large shoot meristeme begins to divide in relation to the gap of an older leaf below (Text-fig. 10), the leaf-trace, now of a squat C-shape and no longer with its xylem in connexion with that of the meristeme, spreads along the left-hand meristeme and that portion of it gradually tapers out. Thereafter, proceeding still farther down the shoot, the remaining portion of the leaf-trace consists of a small semicircular mass on the outer face of the meristeme, the whole being enclosed in a common endodermis but the two ribbons of xylem being quite separate (Text-fig. 11). At various levels leaf-traces from adjacent leaves occasionally become conjoined, not with the meristeme, but with the anomalous leaf-trace (Text-figs. 11 and 12). Finally the leaf-trace decreases in size and tapers out (Text-fig. 12 and Pl. IV, Fig. 9). Somewhat similar developments were also observed in leaf 17.

The factors responsible for these remarkable departures from the normal, which must have taken place during the initial phase of development, are not known. For the present purpose the important point is that although the vascular system of the leaf was well developed as to amount, its spatial disposition and insertion were such as would not on *a priori* grounds be expected to produce a region of stress and therefore a gap in the shoot-stele, and, in point of fact, it has been seen that no leaf-gap developed.

These observations also throw light on the processes which are operative during the initial differentiation of the vascular system of the leaf contemporaneously with that of the shoot. The view has already been outlined (Wardlaw, 1944) that the initial differentiation of vascular tissue in pteridophytes, whether in leaf or shoot, is probably related to the downward diffusion of a substance or substances from the actively growing apical meristem. As this process takes place at approximately the same time in the leaf primordium and in the adjacent shoot tissue, a confluent, coherent, and unified vascular system

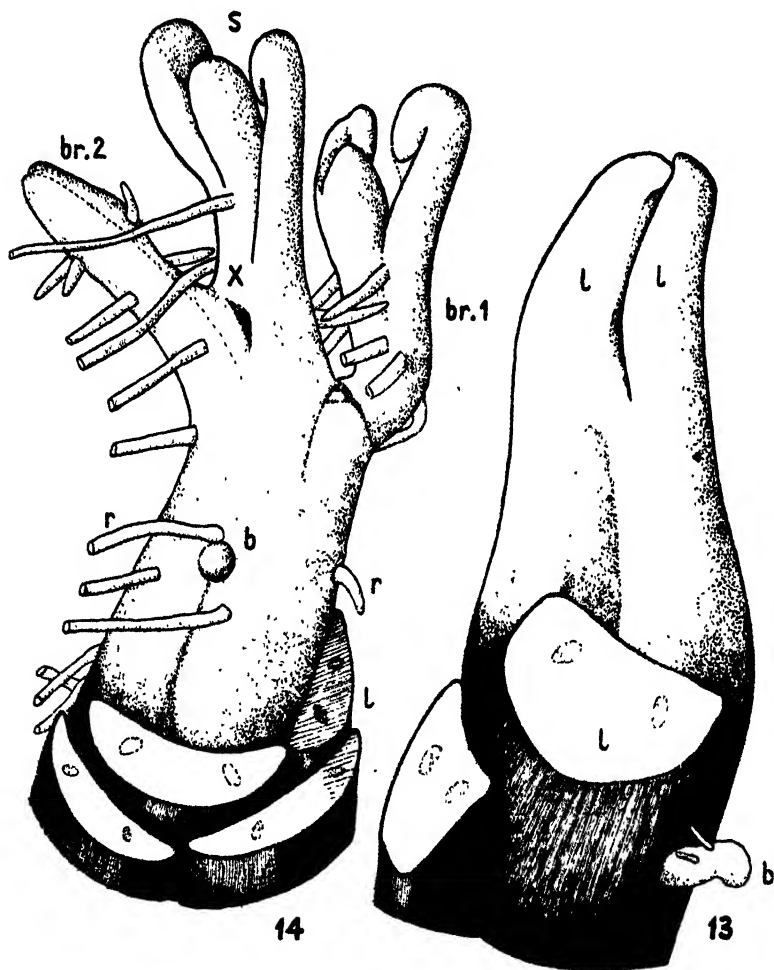
results. The leaf-trace, in fact, 'passes' backwards into the shoot. In support of this hypothesis the evidence from bud development has proved of particular importance. Thus, in *Matteuccia struthiopteris*, *Onoclea sensibilis*,



TEXT-FIGS. 6-12. *Dryopteris filix-mas*. Basipetal series of transverse sections showing the conjunction of an anomalous leaf-trace (*lt*), of leaf 16 (*l 16*) with the shoot stele. Xylem of meristemes (*m*) in solid black; that of leaf-traces cross-hatched; root traces (*rt*) stippled; *lg*, leaf-gap. This series shows that no leaf-gap is associated with the solenostelic leaf-trace of leaf 16 and that the conjunction of leaf-trace and shoot stele is complete at the level of Fig. 9 but incomplete lower down in the shoot. Below the level of Fig. 12 the leaf-trace gradually disappears. *A* and *B* are meristemes which became conjoined at the insertion of the leaf-trace of *l 16*; lower down, the conjoined meristeme divides into two meristemes labelled *A*<sub>1</sub> and *B*<sub>1</sub>. For other details, see Text. (×15.)

and *Dryopteris* spp. buds may be in complete or in incomplete vascular continuity with the shoot-stele, or they may be entirely without connexion. Now, in the data set out above, while the vascular system of the aberrant leaf

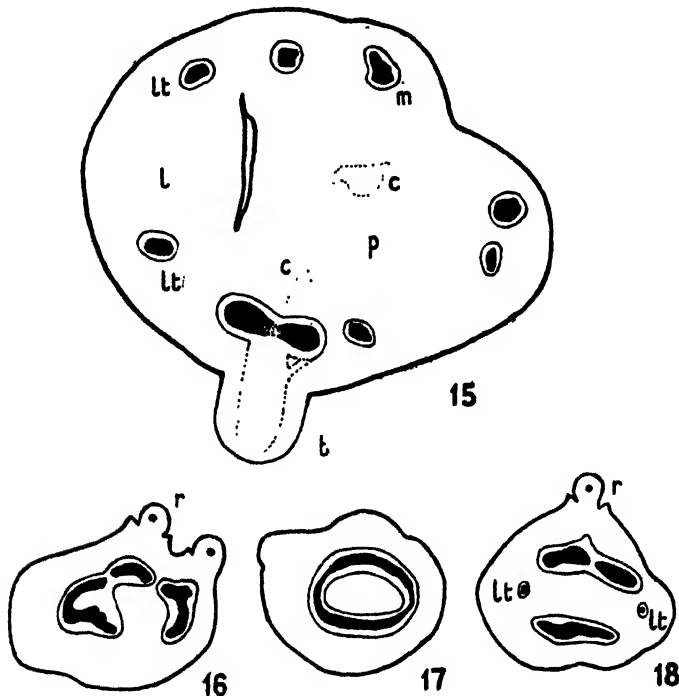
is in complete continuity with the shoot meristele at the level of Text-fig. 9, lower down, i.e. in an older region, the continuity is incomplete, the xylem of the leaf-trace being unconnected with that of the meristele. This relationship is maintained until the leaf-trace eventually tapers out and disappears.



TEXT-FIGS. 13-14. *Onoclea sensibilis*. Fig. 13, apical region of rhizome with older leaf primordia removed. Fig. 14, growth of a rhizome apex, similar to that in Fig. 13, after injection with ammonium nitrate solution. *l*, leaf; *b*, bud; *br*<sub>1</sub>, *br*<sub>2</sub>, lateral branches; *r*, root. ( $\times 3.5$ .)

Moreover, the degree of differentiation of the xylem in the two steles is different. These observations are in close agreement with some that have already been made in certain experimentally induced buds in *Dryopteris filix-mas* (Wardlaw, 1943), where the vascular system of the bud became associated with the shoot-stele, but was incompletely or not conjoined with it.

In the case of the anomalous leaf-traces under consideration the normal time-development relationship of leaf primordium and shoot has apparently been upset, i.e. although the basipetal initial differentiation of the vascular system of the leaf has taken place, it has failed to synchronize completely with that of the shoot-stele. Thus, whereas near the apex a xylem-to-xylem fusion



TEXT-FIGS. 15-18. *Onoclea sensibilis*. Transverse sections of material shown in Fig. 14. Fig. 15, dictyostelic structure, at level of bud (b) in Fig. 13; l, leaf, with two traces; p, pith; m, meristele; lt, leaf-trace; r, roots; c, cavities caused by hypodermic needle. Fig. 16, apparent coalescence of meristemes; Fig. 17, complete solenostele; Fig. 18, dictyostelic condition re-established. ( $\times 8$ .)

can be observed, lower down in the older region the leaf-trace occupies a position on the outside of the meristele. Here mention may be made of the basipetal course of development of the trace in the adventitious leaves of *Cyclamen* described by Boodle (1920).

#### INDUCED SOLENOSTELY IN *ONOCLEA SENSIBILIS*

Stout rhizomes of *Onoclea sensibilis*, defoliated to the extent that a few well-developed leaf primordia still protected the shoot apex, as in Text-fig. 13, were injected below the apex by means of a hypodermic syringe with solutions of ammonium nitrate and asparagin (0.25 per cent.), the control plant being injected with distilled water. A second injection was given 14 days later. The pieces of rhizome were placed on peat in an incubator at 22°-25° C. The



original idea underlying this experiment, which need not be considered in detail here, was to ascertain if any morphological changes took place when the nitrogen supply to the apical meristem was rapidly increased.

In the course of 3–5 weeks the rhizome injected with ammonium nitrate had produced a small lateral bud (*b*) near the point of injection and two strong buds or branches (*br*<sub>1</sub> and *br*<sub>2</sub>) higher up. After an initial period of arrested growth the main shoot (*S* in Text-fig. 14), underwent considerable elongation but was greatly reduced in diameter, no leaves being developed in the immediate region of the two stout branches.

These developments were attended by notable changes in the internal morphology (Text-figs. 15–18). The dictyostelic vascular system in the older region of the shoot (Text-fig. 15) underwent a marked contraction, particularly at level *X*, i.e. above the point of departure of the second branch (*br*<sub>2</sub>, Text-fig. 14): the existing leaf-gaps 'closed' and, in the absence of new leaf development in this region a solenostele was formed. These changes in the configuration of the vascular system are illustrated in Text-figs. 16–18 and Pl. IV, Figs. 10–12. Nearer the apex the dictyostelic condition accompanied the new leaf development.

The rhizome injected with asparagin solution also showed a considerable elongation and decrease in diameter, and in the short leafless region the vascular system consisted of a protostele. This subsequently enlarged, became solenostelic and eventually dictyostelic in relation to the development of new leaves near the apex. The control plant, injected with distilled water, showed incipient bud production and then continued to grow in an approximately normal manner, i.e. as a compact, leafy rhizome. Modifications of the vascular system of the kind indicated above did not take place.

Experiments of this kind are open to various criticisms and, in point of fact, when they were repeated at a different time of the year comparable results were not obtained. But the demonstration that changes can sometimes be induced is nevertheless important because of the indication it affords of a very considerable morphological plasticity in the growing regions of ferns—an observation which may be used to advantage in studies of organization during development. For the present purpose the significant point is that in a fern normally dictyostelic the development of a region of the shoot devoid of leaves has had as a concomitant internal condition the formation of a solenostelic vascular system.

## DISCUSSION

Evidence has been set out which shows conclusively for two species of *Dryopteris* that if the development of leaf primordia is arrested at a sufficiently early stage leaf-gaps are not formed and the vascular system of the shoot develops as an uninterrupted cylinder or solenostele. The experimental data also justify the view expressed by Bower (1923, p. 139) that the shoot-stele is a 'real entity, forming an essential constituent of all axes', and that, in the instances under consideration, it is not merely a composite structure built

up of decurrent leaf-traces. This does not exclude the important contribution which may be made by decurrent leaf-traces in other ferns, for example, those described by Campbell (1921).

During development at the apex, where the several constituent tissues may be considered to be in a plastic condition, the vascular tissue, as Priestley (1928) has pointed out, must be subjected to considerable pressure from the enlarging parenchymatous tissue of both pith and cortex. Provided no other factors intervened to disturb the equilibrium, it might be anticipated that during growth the vascular cylinder in such a radially symmetrical system would show a progressive enlargement and possibly thinning but would not become disrupted. This, in fact, has been borne out in the defoliation experiments, in particular by the series of solenosteles of different sizes of *D. filix-mas* shown in Pl. IV, Figs. 4-6, the development of leaf primordia—normally an equilibrium-disturbing factor—having been precluded. It may be noted in passing that the bilateral symmetry of the leaf primordium permits of a lateral distension and disruption of the vascular tissue in a manner not found in the shoot.

Although the condition of the vascular system described as dictyostely is due, not to the operation of the size factor in the manner envisaged by Bower (1921), but to leaf development, instances are known of disrupted vascular systems which are in no way related to leaf insertion: these are to be found, for example, in the petioles of species of *Dryopteris* and many other ferns, in the 'perforated' dictyosteles of *Stenochlaena* (Bower, 1923) and in the leafless tubers of *Nephrolepis cordifolia* (Sahni, 1916); and in these instances the importance of increasing size as a factor in internal morphology appears to be beyond dispute. A fuller account of these phenomena is given elsewhere (Wardlaw, 1944a).

So far the writer has confined himself to the experimental treatment of species of *Onoclea* and *Dryopteris*. But the ferns as a class possess the great advantage from the experimental point of view of manifesting a very considerable diversity of vascular configuration without the added complication of secondary thickening. Hence the application of the experimental technique described here to such species as *Osmunda cinnamomea*, to mention a single example, may be awaited with interest.

Two further points emerge from the present investigation, namely, that the apices of ferns such as *Dryopteris* and *Onoclea* are not only suitable for experimental studies and capable of withstanding the relatively crude surgery which has been applied to them, but that they also possess considerable morphogenetic plasticity. This information may be used to advantage in experimental investigations of the factors which underlie organization during development.

Lastly, since stelar morphology is profoundly affected by the nature of the leaf development, as well as by the factors operative in the development of the shoot, both sets of factors, but particularly the former, merit the most careful consideration.

## SUMMARY

1. Investigations have been undertaken to ascertain by direct experiment the relation between leaf development and stelar morphology in leptosporangiate ferns, species of *Dryopteris* and *Onoclea* being used for this purpose.

2. It has been shown that if a number of successive leaf-primordia are destroyed at a very early stage, the vascular system of the shoot develops, not as a dictyostele, but as a continuous uninterrupted cylinder or solenostele—a result which also justifies the view that, in the ferns investigated, the shoot-stele is of cauline origin and is not a composite structure composed of decurrent leaf traces.

3. These experiments also throw light on the processes operative in the development of the vascular system of the leaf primordium.

4. The results of related investigations are also considered.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscope preparations and photographic illustrations.

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## DESCRIPTION OF FIGURES IN PLATE IV

Illustrating Professor C. W. Wardlaw's paper on 'Leaf Development and Stelar Morphology'.

(All figures are from untouched photographs.)

Fig. 1. *Dryopteris aristata*. Large experimentally induced solenostele obtained by systematic removal of all young leaf primordia from a growing shoot. ( $\times 7$ .)

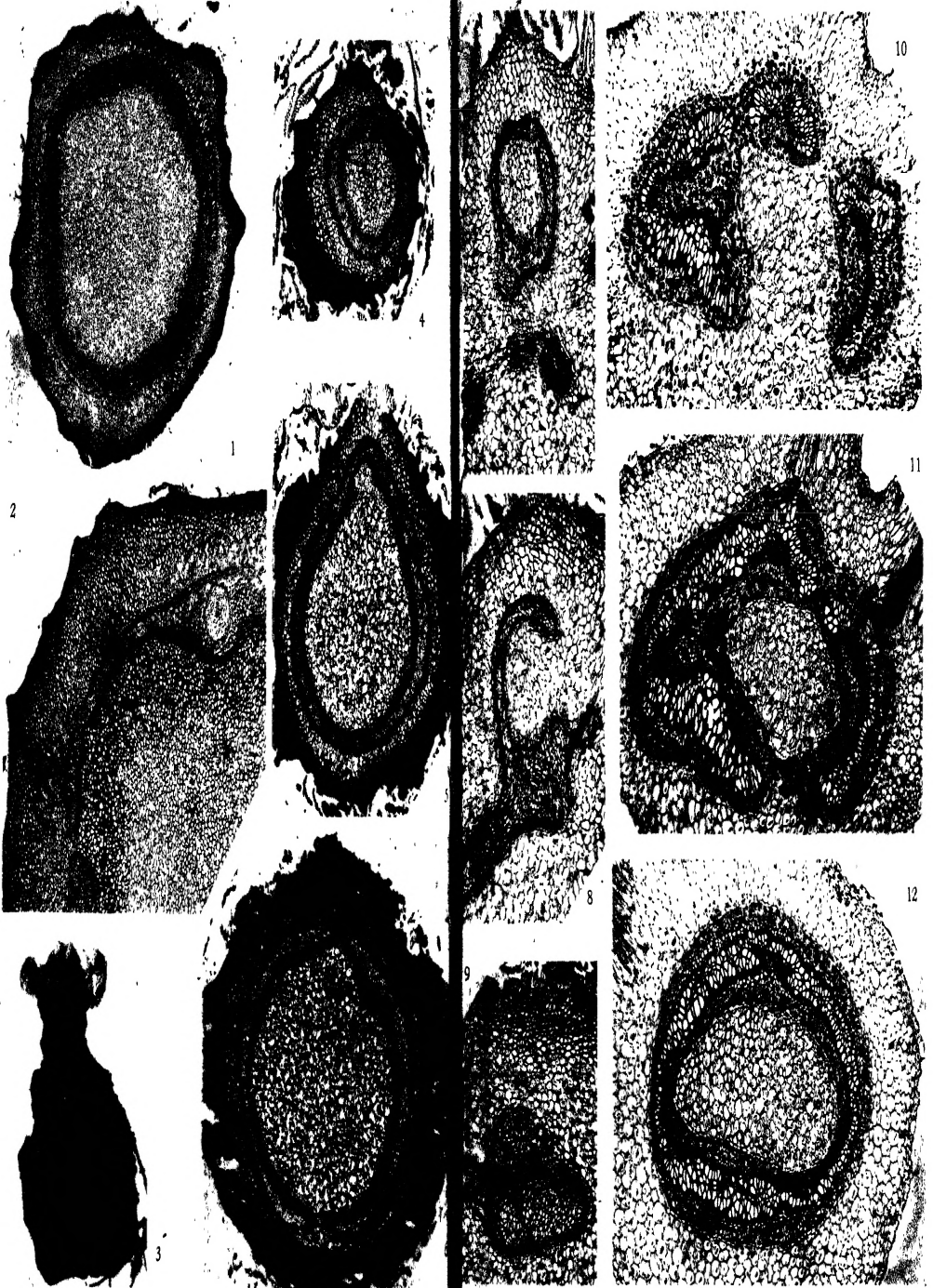
Fig. 2. *Dryopteris aristata*. Leaf-base in an experimentally treated shoot. Instead of five separate leaf-traces, a single more or less continuous trace is present. The shoot-stele (top right) shows an intra-meristele pith with inner endodermis and phloem. ( $\times 18$ .)

Fig. 3. *Dryopteris aristata*. Experimental shoot after several months during which all leaf primordia were systematically removed. Two lateral buds have been allowed to continue growth. ( $\times 1.5$ .)

Figs. 4-6. *Dryopteris filix-mas*. Three sections taken at different successive levels below the apex showing solenostely in an experimentally treated shoot. The leaf-trace in Fig. 5 is seen to be quite undisrupted. ( $\times 25$ .)

Figs. 7-9. *Dryopteris filix-mas*. Three sections in basipetal succession showing the 'insertion' of an anomalous solenostelic leaf-trace on the shoot-stele. In Fig. 9 the leaf-trace, which is tapering out, occupies a position on the outer face of a meristele. ( $\times 35$ .)

Figs. 10-12. *Onoclea sensibilis*. Three sections in acropetal succession showing the development of a solenostele in an experimentally treated dictyostelic shoot. ( $\times 35$ .)





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